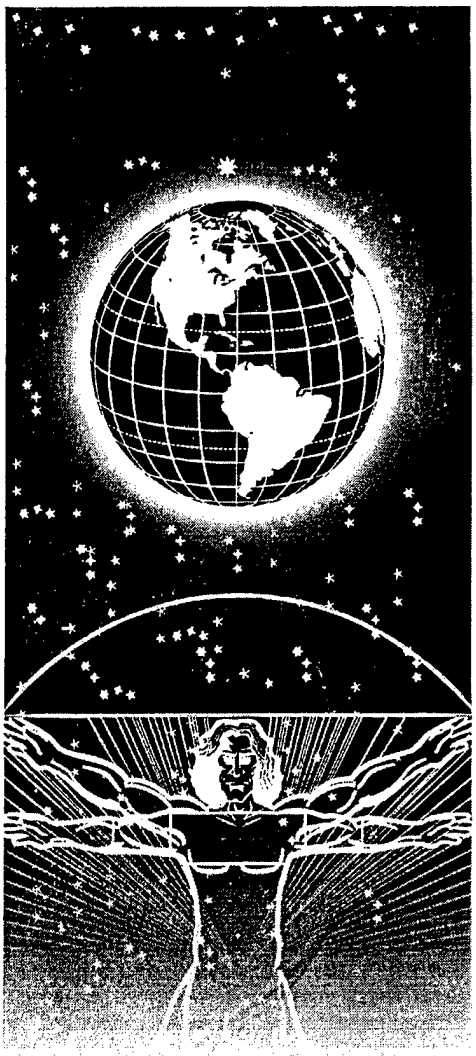
IERA-RS-BR-TR-2000-0003



UNITED STATES AIR FORCE
IERA

Trichloroacetate Tissue Dosimetry and PPAR alpha-Mediated Liver Cancer Induction by Trichloroethylene and Perchloroethylene

Hugh A. Barton

ICF Consulting
K. S. Crump Group
P. O. Box 14348
Research Triangle Park, North Carolina 27709

P. Robinan Gentry
Harvey J. Clewell, III

ICF Consulting
K. S. Crump Group
602 E. Georgia Avenue
Ruston, Louisiana 71270

March 2000

Air Force Institute for Environment, Safety and Occupational Health Risk Analysis
Risk Analysis Directorate
Risk Assessment Division
2513 Kennedy Circle
Brooks Air Force Base TX 78235-5123

NOTICES

When Government drawings, specifications, or other data are used for any purpose other than in connection with a definitely Government-related procurement, the United States Government incurs no responsibility or any obligation whatsoever. The fact that the Government may have formulated or in any way supplied the said drawings, specifications, or other data, is not to be regarded by implication, or otherwise in any manner construed, as licensing the holder or any other person or corporation; or as conveying any rights or permission to manufacture, use, or sell any patented invention that may in any way be related thereto.

The mention of trade names or commercial products in this publication is for illustration purposes and does not constitute endorsement or recommendation for use by the United State Air Force.

The Office of Public Affairs has reviewed this report, and it is releasable to the National Technical Information Service, where it will be available to the general public, including foreign nationals.

This report has been reviewed and is approved for publication.

Government agencies and their contractors registered with Defense Technical Information Center (DTIC) should direct requests for copies to: Defense Technical Information Center, 8725 John J. Kingman Rd., STE 0944, Ft. Belvoir, VA 22060-6218.

Non-Government agencies may purchase copies of this report from: National Technical Information Services (NTIS), 5285 Port Royal Road, Springfield, VA 22161-2103.

Elizabeth A. Maull

Elizabeth A. Maull, Ph.D., GS-13
Toxicologist, RSRE

Kenneth L. Cox

Kenneth L. Cox, Lt Col, USAF, MC, SFS
Chief, Risk Assessment Division

REPORT DOCUMENTATION PAGE	Form Approved OMB No. 0704-0188

1. AGENCY USE ONLY *(Leave blank)*	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	15 March 2000	Final, Sep 98 - Mar 00

4. TITLE AND SUBTITLE
Trichloroacetate Tissue Dosimetry and PPAR alpha-mediated Liver Cancer Induction by Trichloroethylene and Perchloroethylene

5. FUNDING NUMBERS

6. AUTHOR(S)
Barton, Hugh A., Gentry, P. Robinan, Clewell, Harvey J., III

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
K.S. Crump Group, Inc.
ICF Kaiser International
P.O. Box 14348
Research Triangle Park, NC 27709

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
AFIERA/RSRE
2513 Kennedy Circle
Brooks AFB TX 78235-5123

10. SPONSORING/MONITORING AGENCY REPORT NUMBER
IERA-RS-BR-TR-2000-0003

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION AVAILABILITY STATEMENT
Approved for public release; distribution unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200 words)*

A biologically-motivated dose-response analysis was undertaken for liver cancer induction in mice by trichloroethylene (TCE) and perchloroethylene (PERC). The mechanistic hypothesis evaluated was whether tumors resulted from a peroxisome proliferator-activated receptor-alpha (PPARa)-mediated process initiated by formation of trichloroacetate (TCA) from the two volatile organics. Available mechanistic data for TCE, PERC, and TCA were compared with those for prototypical PPARa-ligands. Several early and late events were consistent: increased liver to body weight ratio (LW/BW) due to induction of peroxisomes, hypertrophy, and cell proliferation; the phenotype of induced foci; and reversibility of tumor response with cessation of exposure. One difference was hepatic accumulation of lipid with PERC, but not the other compounds. Use of precursor events for analyzing cancer dose response was evaluated; increased LW/BW was suggested as a useful indicator of the pleiotropic response necessary for PPARa-mediated liver carcinogenesis. Physiologically based pharmacokinetic models provided estimates of internal dose metrics for TCA. Oral and inhalation studies for LW/BW and cancer were evaluated to obtain points of departure. Low dose extrapolation used a margin of exposure approach for TCE; TCA and PERC were not evaluated. While there are difficulties in analyzing the hypothesis due to the variety of exposure protocols used in the studies with TCE, PERC, and TCA, this analysis indicated substantial consistencies in the database supporting a PPARa-mediated process for TCE- and TCA-induced liver carcinogenesis as a major causative process.

14. SUBJECT TERMS	15. NUMBER OF PAGES
trichloroethylene, trichloroacetate, perchloroethylene, biologically-based dose response model, PPAR-alpha	72
	16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	UL

THIS PAGE INTENTIONALLY LEFT BLANK

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

TRICHLOROACETATE TISSUE DOSIMETRY AND PPARα-MEDIATED LIVER CANCER INDUCTION BY TRICHLOROETHYLENE AND PERCHLOROETHYLENE

TECHNICAL SUMMARY

A biologically directed dose-response analysis was undertaken for liver cancer induction in mice by trichloroethylene (TCE) and perchloroethylene (PERC). The mechanistic hypothesis evaluated was that the tumors resulted from a peroxisome proliferator-activated receptor (PPARα)-mediated process initiated by formation of trichloroacetate (TCA) from the two volatile organics. An extensive literature review compared the available mechanistic information for TCE, PERC, and TCA with that for prototypical pharmaceutical PPARα-ligands. Several early and late events were consistent among these ligands including: increases in liver to body weight ratio (LW/BW) due to induction of peroxisomes, hypertrophy, and cell proliferation; the phenotype of induced foci; and reversibility of tumor response with cessation of exposure to PPARα-ligands. A notable difference observed with PERC was the induction of lipid accumulation in liver, which appears to be associated with the parent compound since it was not observed with TCE, TCA, or other PPARα-ligands. The use of precursor events for analyzing cancer dose response was evaluated; increased LW/BW was found to be a useful indicator of the pleiotropic response necessary for PPARα-induced liver carcinogenesis. Physiologically based pharmacokinetic models provided estimates of internal dose metrics for TCA, particularly the area under the curve (AUC). Both oral and inhalation studies were included in the analysis. Points of departure were then obtained for hepatocarcinogenicity and increased LW/BW induced by TCE, PERC, or TCA. Low dose extrapolation using a margin of exposure approach was undertaken with the points of departure obtained for TCE. Similar analyses were not undertaken for TCA-induced liver cancer due to the lack of a lifetime study in male mice, which are more sensitive than females. Finally, the involvement of multiple mechanisms in PERC-induced carcinogenesis indicated that use of a dose metric for TCA might not be appropriate. There were difficulties in analyzing the hypothesis of a PPARα-mediated process due to the variety of different exposure protocols used in the studies with TCE, PERC, and TCA. None-the-less, a careful analysis indicates that there are substantial consistencies in the database that support a PPARα-mediated process for TCE- and TCA-induced liver carcinogenesis.

INTRODUCTION

Liver tumors in mice resulting from trichloroethylene (TCE) and perchloroethylene (PERC) exposure have long been a major focus for the cancer risk assessment of these compounds (EPA, 1985; Bogen, 1988; Clewell, 1995; ATSDR, 1996; ATSDR, 1997b). While it has been known that TCE, PERC, and their major stable metabolite, trichloroacetate (TCA) induced the proliferation of peroxisomes, the relevance of this finding to liver carcinogenesis has long been debated (Ashby *et al.*, 1994; Fenner-Crisp, 1996). As a consequence, this mode of action has not formed the basis for regulatory dose-response assessments for these compounds (Cogliano, 1999). Existing regulatory analyses concluded that the mode of action giving rise to the liver cancers was unclear, so it should be assumed that metabolites of the two compounds acted as genotoxic agents causing the tumors (EPA, 1985). Major advances have occurred in the scientific understanding of the liver carcinogenicity of compounds that cause peroxisome proliferation, particularly the role of the peroxisome proliferator-activated receptor-α (PPARα) and resulting alterations in cell cycling (Bocos *et al.*, 1995; Fenner-Crisp, 1996; Roberts, 1996; Roberts *et al.*, 1997; Cattley *et al.*, 1998; Gonzalez *et al.*, 1998). Therefore, it is appropriate to evaluate the evidence that TCE and PERC induce liver cancer in mice through a PPARα-mediated mode of action and, if strongly supported, conduct a dose-response analysis for these cancers based upon that mode of action.

Traditionally TCE, PERC, clofibrate, WY-14,643, and other compounds that induce proliferation of peroxisomes have been referred to as peroxisome proliferators (Ashby *et al.*, 1994). While the role of peroxisomal proliferation in liver carcinogenesis has been controversial, activation of PPARα is required for the cancer response (Peters *et al.*, 1997b). Therefore, in this paper, these compounds are referred to as PPARα-ligands to stress this activity and its importance in their dose-response assessment. However, it is also important to note that some PPARα-ligands, such as diethylhexylphthalate, cause other biological effects through processes independent of PPARα (Ward *et al.*, 1998).

Both TCE and PERC have also been shown to induce cancers at sites other than the liver. Traditionally, findings of cancers at multiple sites were considered to provide stronger evidence that the carcinogenicity in animals was relevant to humans (EPA, 1986). This would certainly be

the case if the underlying mechanism of action were the same in each tissue, for example formation of mutagenic DNA adducts. However, it is widely accepted that TCE and PERC have complex metabolic pathways (Bruckner *et al.*, 1989; Goeptar *et al.*, 1995) (Dekant *et al.*, 1989; Vamvakas *et al.*, 1993) and the resulting tumors arise from specific metabolites and tissue-specific modes of action (Clewell *et al.*, 1995; Goeptar *et al.*, 1995; Clewell *et al.*, 1999). The discussions provided here are specifically an analysis of the liver cancers observed only in mice. The issues raised by other tumors, notably the kidney tumors observed in rats and humans (NTP, 1983; NTP, 1988; Bruning *et al.*, 1996; Bruning *et al.*, 1997; Vamvakas *et al.*, 1998) that arise from glutathione conjugation of TCE (Dekant *et al.*, 1986; Dekant *et al.*, 1987; Dekant *et al.*, 1990), are substantially different and must be addressed separately.

LIVER CANCER INDUCTION BY TCE, PERC AND METABOLITES

Trichloroethylene and PERC both cause liver cancer in the mouse, but not the rat (NCI, 1976; NCI, 1977; NTP, 1983; Maltoni *et al.*, 1986; NTP, 1986; Maltoni *et al.*, 1988; NTP, 1988) (Table 1). Studies have also been carried out with several metabolites of TCE and PERC, including chloral and TCA (Table 1). These studies have also found liver cancer only in mice and not in rats (DeAngelo and Daniel, 1990; Daniel *et al.*, 1992; DeAngelo and George, 1995; Pereira, 1996; DeAngelo *et al.*, 1997). Thus, qualitatively the cancer data are all consistent with TCA acting as the ultimate carcinogenic metabolite of TCE, PERC, and chloral.

A potentially confounding factor in evaluting this issue is the use of corn oil in all the oral cancer bioassays with TCE and PERC, while those with TCA have used aqueous solutions. Corn oil has been shown to significantly increase the weak peroxisome proliferation response induced by TCA in rats, although no equivalent data are available for mice (DeAngelo *et al.*, 1989). Small, statistically significant, or no increases in hepatotoxicity occurred in mice exposed to TCE for four weeks by either corn oil or aqueous (Emulphor) gavage (Merrick *et al.*, 1989). To eliminate the possible confounding influence of corn oil, the results of inhalation studies would be preferable for estimating cancer risk. Pharmacokinetic modeling would then be used for route-to-route extrapolation to estimate oral risks based upon the inhalation studies. However, many of the inhalation studies involved less than lifetime exposures limiting the utility of this option (Maltoni *et al.*, 1986).

TABLE 1: SUMMARY OF MOUSE BIOASSAY RESULTS FOR TCE, TCA, AND PERC

Study	Strain	Sex	Duration	Average Administered Dose[a] (mg/kg/d)	Endpoint	Incidence/Significance of Cancer
			TRICHLOROETHYLENE — GAVAGE			
(NCI, 1976)	B6C3F1	M	5d/w, 90w	0, 1013[b], 2027[c]	Hepatocellular carcinoma	1/20, 26/50*, 31/48*
		F	5d/w, 90w	0, 753[d], 1597[e]	Hepatocellular carcinoma	0/20, 4/50, 11/47*
(NTP, 1990)	B6C3F1	M	5d/w	0, 1000	Hepatocellular carcinoma	8/48, 31/50*
			103w		Hepatocellular adenoma & carcinoma	14/48, 39/50*
		F	5d/w	0, 1000	Hepatocellular carcinoma	2/48, 13/49*
			103w		Hepatocellular adenoma & carcinoma	6/48, 22/49*
(Van Duuren *et al.*, 1979)	Swiss	M/F	1d/w, 622d	0, 17[f]	—	*NS*
(Henschler, 1984)	ICF Swiss	M	5d/w, 18m	0, 2400[g]	—	*NS*
		F	5d/w, 18m	0, 1800[h]	—	*NS*

* Statistically significant (p ∃0.05); *SS* - Statistically significant; *NS* - Not statistically significant for any dose group.

[a] Average dose administered from start of dosing until determination of cancer in animal.
[b] Based on administration of 1000 mg/kg for 12 weeks, 1200 mg/kg for 66 weeks, and no treatment for 12 weeks.
[c] Based on administration of 2000 mg/kg for 12 weeks, 2400 mg/kg for 66 weeks, and no treatment for 12 weeks.
[d] Based on administration of 700 mg/kg for 12 weeks, 900 mg/kg for 66 weeks, and no treatment for 12 weeks.
[e] Based on administration of 1400 mg/kg for 12 weeks, 1800 mg/kg for 66 weeks, and no treatment for 12 weeks.
[f] Based on administration of 0.5g per mouse (assumed 0.03 kg) for 622 days (89weeks)
[g] Based on administration of 2400 mg/kg for 18 months and no treatment for 28 weeks.
[h] Based on administration of 1800 mg/kg/day (assumed 0.03 kg) for 622 days (89 weeks).

TABLE 1 (cont.)

Study	Strain	Sex	Duration	Exposure Concentration (ppm)	Endpoint	Incidence/Significance of Cancer
			TRICHLOROETHYLENE — INHALATION			
(Bell, 1978)	B6C3F1	M	6h/d, 5d/w, 104w	0, 100, 300, 600	Hepatocellular carcinoma	18/99, 28/95*,31/100*, 43/97*
					Hepatocellular adenoma & carcinoma	20/99, 35/95*, 38/100*, 53/93*
		F	6h/d, 5d/w, 104w	0, 100, 300, 600	Hepatocellular adenoma & carcinoma	8/99, 9/100, 10/94, 17/99*
(Henschler *et al.*, 1980)	HAN: NMRI	M	6h/d, 5d/w, 18m	0, 100, 500	—	*NS*
		F	6h/d, 5d/w, 18m	0, 100, 500	Malignant lymphoma	*SS*
(Fukuda *et al.*, 1983)	ICR	F	7h/d, 5 /w, 104w	0, 50, 100, 450	Lung adenocarcinoma	*SS*
(Maltoni *et al.*, 1986; Maltoni *et al.*, 1988)	Swiss (BT 305)	M	7h/d, 5d/w, 78w	0, 100, 300, 600	Hepatomas	4/90, 2/90, 8/90, 13/90*
					Pulmonary tumors (mostly benign)	*SS*
		F	7h/d, 5d/w, 78w	0, 100, 300, 600	—	*NS*
	B6C3F1 (BT 306 bis)	M	7h/d, 5d/w, 78w	0, 100, 300, 600	—	*NS*
		F	7h/d, 5d/w, 78w	0, 100, 300, 600	Pulmonary tumors (almost exclusively benign)	*SS*
	B6C3F1 (BT 306)	M/F	7h/d, 5d/w, 78w	0, 100, 300, 600	Hepatomas	4/180, 5/180, 7/180, 15/180*

* Statistically significant ($p \exists 0.05$); *SS* - Statistically significant; *NS* - Not statistically significant for any dose group.

TABLE 1 (cont.)

Study	Strain	Sex	Duration	Water Concentration (g/L)	Endpoint	Incidence/Significance of Cancer
				TRICHLOROETHYLENE — DRINKING WATER		
(Herren-Freund *et al.*, 1987)	B6C3F1	M	61w	0, 0.04	Hepatocellular carcinoma	0/22, 3/32
				CHLORAL HYDRATE — DRINKING WATER		
(Daniel *et al.*, 1992)	B6C3F1	M	104w	0, 1	Hepatocellular carcinoma	2/20, 11/24*
					Hepatocellular adenoma & carcinoma	3/20, 17/24*
				TRICHLOROACETIC ACID — DRINKING WATER		
(Bull *et al.*, 1990)	B6C3F1	M/F	52w	0, 1, 2	—	*NS*
(Ferreira-Gonzalez *et al.*, 1995)	B6C3F1	M	104w	0, 4.5	Hepatocellular carcinoma	19%, 73.3%
(Herren-Freund *et al.*, 1987)	B6C3F1	M	61w	0, 5	Hepatocellular carcinoma	0/22, 7/22*
					Hepatocellular adenoma	2/22, 8/22*
(Pereira, 1996)	B6C3F1	F	360d	0, 0.26, 0.88, 2.6	Hepatocellular carcinoma	0/40, 0/40, 0/19, 5/20*
		F	576d	0, 0.26, 0.88, 2.6	Hepatocellular adenoma	2/90, 4/53, 3/27, 7/18*
					Hepatocellular carcinoma	2/90, 0/53, 5/27*, 5/18*

* Statistically significant ($p \exists 0.05$); *SS* - Statistically significant; *NS* - Not statistically significant for any dose group.

TABLE 1 (cont.)

Study	Strain	Sex	Duration	Average Administered[a] Dose (mg/kg/d)	Endpoint	Incidence/Significance of Cancer
PERCHLOROETHYLENE — GAVAGE						
(NCI, 1977)	B6C3F1	M	5d/w, 90w	0, 536[j], 1072[k]	Hepatocellular carcinoma	2/20, 32/49*, 27/48*
		F	5d/w, 90w	0, 386[l], 772[m]	Hepatocellular carcinoma	0/20, 19/48*, 19/48*
PERCHLOROETHYLENE — INHALATION						
(NTP, 1986)	B6C3F1	M	6 h/d, 5d/w, 103w	0, 100, 200	Hepatocellular carcinoma	7/49, 25/49*, 26/50*
					Hepatocellular adenoma & carcinoma	17/49, 31/49*, 41/50*
		F	6 h/d, 5d/w, 103w	0, 100, 200	Hepatocellular carcinoma	1/48, 13/50*, 36/50*
					Hepatocellular adenoma & carcinoma	4/48, 17/50*, 38/50*

* Statistically significant (p ∃0.05); *SS* - Statistically significant; *NS* - Not statistically significant for any dose group.

[a] Average dose administered from start of dosing until determination of cancer in animal.

[j] Based on administration of 450 mg/kg for 11 weeks, 550 mg/kg for 67 weeks, and no treatment for 12 weeks.

[k] Based on administration of 900 mg/kg for 12 weeks, 1100 mg/kg for 66 weeks, and no treatment for 12 weeks.

[l] Based on administration of 300 mg/kg for 12 weeks, 400 mg/kg for 66 weeks, and no treatment for 12 weeks.

Based on administration of 600 mg/kg for 12 weeks, 800 mg/kg for 66 weeks, and no treatment for 12 weeks.

TCE, PERC, AND PPARα-MEDIATED LIVER CANCER

Trichloroethylene and PERC activate PPARα due to the production of the stable metabolite, TCA (Ashby *et al.*, 1994; Zhou and Waxman, 1998). We will evaluate the evidence for a PPARα-mediated mode of action for mouse liver carcinogenesis using studies with TCE, PERC, and TCA. None of these compounds are used as prototypic PPARα-ligands in mechanistic research on PPARα-mediated effects because they elicit weaker responses at higher doses than is the case for pharmaceutical compounds or diethylhexylphthalate (Ashby *et al.*, 1994). Thus, the data for TCE, PERC, and TCA, discussed below, demonstrate that they act through PPARα, but data from the prototypical compounds (*e.g.* WY-14,364, clofibrate, nafenopin, *etc.*) are required to more completely describe the mode of action leading to liver carcinogenesis.

A critical question in the field of TCE and PERC carcinogenicity has been the relative importance of TCA and dichloroacetate (DCA) as active metabolites because they have significantly different modes of action (Latendresse and Pereira, 1997; Stauber and Bull, 1997). Resolution of this question has been hindered by artifactual production of DCA from TCA in analytical chemistry methods (Ketcha *et al.*, 1996; Merdink *et al.*, 1998). Mice had been reported to produce significant levels of DCA from TCE, while little or no DCA was detectable in samples from rats and humans (Larson and Bull, 1992b; Templin *et al.*, 1993; Bruning *et al.*, 1998). Absent controls to demonstrate there is no interconversion of the metabolites during the analytical processes, all reports of DCA production from TCE must be treated with caution. Recent data from mice demonstrated that production of DCA is at or below detection limits and that DCA cannot account for the carcinogenicity of TCE (Merdink *et al.*, 1998; Barton *et al.*, 1999). Therefore, the remainder of our analysis focuses only upon TCA, though there remains the possibility that low levels of DCA modulate the development of mouse liver tumors from TCE and PERC, so they might represent a variant of a pure PPARα-mediated process.

Direct activation of PPARα by TCA was demonstrated using heterologous expression of PPARα and a reporter gene (Issemann and Green, 1990; Zhou and Waxman, 1998). High concentrations of TCA were required for the activation as compared to other ligands (Issemann and Green, 1990). Trichloroethylene did not activate PPARα, presumably due to inability of the cells to

metabolize it to TCA (Zhou and Waxman, 1998); no data are available for PERC, but the results would be expected to depend upon metabolism to TCA.

Several early biological effects mediated by PPARα have been observed with exposure to TCE, PERC, and TCA. The most widely studied has been increased liver weight (LW), usually expressed as liver weight to body weight ratio (LW/BW), arising from peroxisomal proliferation, cellular hypertrophy, and cell proliferation (Barton and Das, 1996; Barton and Clewell, 1998). These changes in LW/BW are reversible with TCE and PERC (Kjellstrand *et al.*, 1981; Kjellstrand *et al.*, 1984) as commonly occurs for PPARα-ligands (Roberts *et al.*, 1995). Other endpoints have been examined in liver including induction of CYP4A (ω-hydroylase activity), peroxisome proliferation (as determined by enzymatic, histological or morphometric analyses) and changes in DNA synthesis (measured by [^{3}H]thymidine uptake or bromodeoxyuridine (BrDU) labeling) and content (decreases indicating cellular hypertrophy). Evidence for CYP4α induction and peroxisome proliferation will be reviewed first as these are specific indicators of PPAR-mediated activity. Data on cell proliferation, as a measure directly relevant to the carcinogenic process, will then be considered followed by evidence for similarities in tumor promotion between TCA and other PPARα-ligands.

CYP4A induction is a classic indicator of PPARα-mediated activity in rodents (Gibson, 1996; Johnson *et al.*, 1996a; Johnson *et al.*, 1996b; Simpson, 1997). The CYP4A isoforms are species specific, with 4A1,4A2, and 4A3 in rats and 4A11 the major ω-hydroxylase in humans (Kimura *et al.*, 1989; Powell *et al.*, 1996). *CYP4A* genes contain peroxisome proliferator response elements (PPREs) that bind heterodimers of PPARα and the retinoid X receptor (Johnson *et al.*, 1996a; Johnson *et al.*, 1996b). Dose-dependent increases in CYP4A mRNA occurred from day 2 to 56 in mice exposed to TCE by corn oil gavage (Geiss *et al.*, 1997). Large increases in mRNA were observed at 400 mg/kg/day with some dose-dependent increase up to 1200 mg/kg/day. Trichloroacetate induced ω-hydroylase activity and immunodetectable CYP4A (Austin *et al.*, 1995; Parrish *et al.*, 1996; Zanelli *et al.*, 1996), but not P450 2E1 indicating specificity of the CYP4A response.

Increases in peroxisomes and peroxisomal enzymes (*e.g.*, palmitoyl-CoA oxidase) were observed in mice exposed to TCE or PERC (Elcombe, 1985; Elcombe *et al.*, 1985; Goldsworthy and Popp, 1987; Odum *et al.*, 1988; Channel *et al.*, 1998). Increases in palmitoyl-CoA oxidation following 10 daily doses of 1000 mg/kg/day TCE and PERC were greater in the male mouse than the rat (4 and 6-fold versus less than 2-fold) (Goldsworthy and Popp, 1987), while smaller increases were seen by inhalation (Odum *et al.*, 1988). A greater difference in palmitoyl-CoA oxidation was seen in mice versus rats (8- vs. 1.1-fold increases, respectively) exposed to 1000 mg/kg/day TCE for 10 days (Elcombe *et al.*, 1985). A dose-response study again found much greater response in mice following 10 daily doses (Elcombe, 1985). No increase in palmitoyl-CoA oxidation was observed at 50 mg/kg/day, while a dose-dependent increase occurred from 100 - 2000 mg/kg/day. Peroxisomal proliferation also occurred in response to TCA exposure (Elcombe, 1985; Goldsworthy and Popp, 1987; DeAngelo *et al.*, 1989; Styles *et al.*, 1991; Austin *et al.*, 1995). The study of DeAngelo *et al.* (1989) shows four strains of mice (including B6C3F1) to be more responsive to TCA than two strains of rats (Osborne-Mendel and F344). Goldsworthy and Popp (1987) reports equal sensitivity of rats and mice, while Elcombe *et al.* (1985) report greater sensitivity in rats. These latter two studies dosed TCA in corn oil, which DeAngelo *et al.* (1989) show doubles the response in rats.

Histologically, studies with TCE and PERC consistently show increases in peroxisomes in the centrilobular region (Elcombe *et al.*, 1985; Odum *et al.*, 1988; Channel *et al.*, 1998). This is consistent with CYP4A induction and peroxisomal proliferation observed with well-characterized PPARα-ligands, such as clofibrate (Bell *et al.*, 1991; Bars *et al.*, 1993; Lindauer *et al.*, 1994; Chen *et al.*, 1995; Fahimi *et al.*, 1996; Beier *et al.*, 1997).

Overall, these data demonstrate that PPARα is activated by *in vivo* exposure to TCE and PERC, and that TCA is a likely candidate for this activity. Furthermore, there is data indicating that corn oil increases the degree of response in rats; similar data are lacking for mice.
Alterations in cell proliferation have been measured following exposure to TCE, PERC, and TCA. A peak of cell proliferation, as measured by proliferating cell nuclear antigen (PCNA) immunohistochemistry, was observed at 10 days in mice dosed with 1200 mg/kg/day TCE by corn oil gavage (Channel *et al.*, 1998). Small increases in proliferation were observed at lower

doses on this day and no increases were observed at earlier or later timepoints at any dose. Increases in proliferation also were reported to be panlobular.

Increased DNA synthesis measured by [^{3}H]thymidine uptake was also reported to be dose-dependent following 10 daily corn oil gavage doses of TCE in two strains of mice, but not rats (Elcombe *et al.*, 1985; Dees and Travis, 1993). Increases in mitotic figures were also observed in mice (Dees and Travis, 1993). Increases in DNA synthesis have been reported with 3 days of TCE exposure and inconsistently at 3 weeks (Stott *et al.*, 1982). Increases in liver DNA synthesis have also been reported following PERC exposures (Schumann *et al.*, 1980). Increased hepatocyte proliferation begins following a few days of dosing with TCA, continues for a short period of time, and then ceases. Aqueous gavage of TCA elevated DNA synthesis after 3 daily exposures (Styles *et al.*, 1991). Trichloroacetate in drinking water elevated the BrdU-labeling index in mice treated for 5 days, but not at 12 or 33 days (Pereira, 1996). Increased DNA synthesis ([^{3}H]thymidine uptake) was observed with 2.0 g/L TCA in drinking water at 5 and 14, but not 2 days (Sanchez and Bull, 1990). Corn oil gavage with TCA resulted in a dose-dependent increase in liver DNA synthesis following 10 daily doses in both male and female mice (Dees and Travis, 1994). A longer study of TCA in drinking water showed increases in the BrdU-labeling index at 14 and 28 days (Stauber and Bull, 1997). Suppression of proliferative activity was observed at 350 days TCA exposure in normal hepatocytes relative to control animals, but not in cells in altered hepatic foci or tumors (Stauber and Bull, 1997).

Finally, *in vitro* studies have demonstrated altered cell cycling characteristics of mouse, but not rat, hepatocytes with exposure to TCA (James and Roberts, 1994; Stauber *et al.*, 1998). These data are consistent with the observations for most PPARα-ligands, which show an early burst of cell proliferation (Marsman *et al.*, 1992; Roberts, 1996; Roberts *et al.*, 1997). A few PPARα-ligands, notably WY14,643, sustain cell proliferation which correlates with the much greater potency and more rapid tumor development observed with these compounds (Marsman *et al.*, 1988; Marsman *et al.*, 1992).

Recent mode-of-action studies have focussed upon the activity of TCA because it is itself an important disinfection byproduct of drinking water chlorination as well as a common metabolite

of TCE and PERC. Mice exposed to TCA in drinking water for 38 or 50 weeks developed altered hepatic foci and tumors (Stauber and Bull, 1997). These foci were predominantly large, did not express *c-Jun* or *c-Fos*, and almost exclusively stained basophilic. Basophilic, glutathione S-transferase-π (GST-π) negative tumors were also observed in female mice exposed to TCA in drinking water for 12 or 19 months and in tumor promotion protocols using N-methyl-N-nitrosourea initiation in combination with TCA (Pereira, 1996; Pereira *et al.*, 1997). Reversibility of proliferative lesions has been observed with cessation of TCA exposure (Bull *et al.*, 1990).

These findings are consistent with those for WY-14,643 and nafenopin (Kraupp-Grasl *et al.*, 1991; Marsman and Popp, 1994). Studies with classic PPAR-ligands have shown that the phenotype of foci induced by those ligands is basophilic and GST-π negative (Rao and Reddy, 1996). Importantly, these studies have also demonstrated that the activity of the PPARα-ligands is that expected for tumor promotion. They promote preexisting basophilic lesions resulting either from chemical treatment or aging (Kraupp-Grasl *et al.*, 1991). Cessation of the exposure results in reductions in tumor size and number (Kraupp-Grasl *et al.*, 1990; Cattley *et al.*, 1991; Kraupp-Grasl *et al.*, 1991; Grasl-Kraupp *et al.*, 1997). Since basophilic foci are a small subset of all spontaneous lesions, the oncogene expression patterns expected from promoting these lesions would not necessarily be the same as that observed for all spontaneous lesions. Thus, gene mutation patterns different from the average spontaneous pattern do not necessarily indicate genotoxic activity of a chemical (Maronpot *et al.*, 1995; Melnick *et al.*, 1996).

In summary, there is a substantial database demonstrating that TCE, PERC, and TCA activate PPARα leading to alterations in liver characteristic of prototypical PPARα-ligands. These include specific short-term responses such as CYP4A induction and peroxisome proliferation. Alterations in cell cycling, notably a burst of cell proliferation are observed. In longer duration studies, neoplastic lesions resulting from TCA exposure have been shown to have similar characteristics (*i.e.* basophilic, GST-π negative) as prototypical PPARα-ligands, such as nafenopin.

Before considering the implications for liver cancer dose-response analysis of a PPARα-mediated mode of action, a final question should be addressed: whether there is credible evidence for other modes of action. The three major hypotheses that have been proposed are genotoxicity, cytotoxicity, and a mode-of-action dependent upon DCA (EPA, 1985; Bogen and Gold, 1997). None of these currently appear particularly plausible alternatives to the PPARα-mediated activity of TCA.

Genotoxicity might arise from one of several metabolites of TCE or PERC - epoxides or chloral formed by P450 2E1 activity and metabolites of the cysteine derivatives formed by glutathione conjugation. Overall, the evidence for TCE and PERC indicate little or no genotoxicity (ATSDR, 1997a). While there is evidence for formation of epoxide from TCE, it appears that substantial rearrangement occurs in the active site of the enzyme resulting in release of chloral and little epoxide (Miller and Guengerich, 1982; Miller and Guengerich, 1983). If substantial epoxide formation occurred, TCE and PERC would be expected to cause liver cancer in both rats and mice as was observed with vinyl chloride. Single strand breaks were reported in mice but, a comparative study indicates that if TCE is active it has only very weak mutagenic activity at high doses (Walles, 1986). Chloral has been shown to induce genotoxicity, particularly chromosomal damage, but very high doses are required (Ferguson *et al.*, 1993) making it appear unlikely that this mechanism is responsible for TCE-induced carcinogenesis. Chloral also is metabolized to TCA, so one possible hypothesis would be that the PPARα-mediated activity, in part, promotes damage caused by the aldehyde. Previously, dosimetric analysis suggested that TCA alone could account for the liver tumors observed with TCE (Clewell *et al.*, 1994). Studies in PPARα-knock out (PPAR -/-) mice may be the only way to resolve the issue (Gonzalez, 1997; Gonzalez *et al.*, 1998). It is unknown if the parental strain used to create these mice would be responsive to TCE or PERC induced liver cancer, as there is some strain specificity to the liver cancer response (See Table 1). Finally, the glutathione-conjugation pathway produces metabolites that are genotoxic *in vitro*. This pathway is predominantly dependent upon β-lyase activity in the kidney, so it appears unlikely to play a major role in liver toxicity.

While a very minor contribution of genotoxicity from one of these TCE and PERC metabolites to the liver cancers cannot be categorically excluded, it is instructive to consider the evidence

with WY-14,643 and the PPARα-knock out mice. It has been argued that the limited evidence of genotoxicity with WY-14,643 should not be ignored when evaluating its liver carcinogenicity (Melnick *et al.*, 1996). However, the incidence of liver tumors and precursor hepatic alterations in the PPARα (-/-) mice was reduced to zero from 100% in the PPARα (+/+) parental strain (Peters *et al.*, 1997b). This study used small numbers of animals (9 per group) so it cannot rule out a small genotoxic component of the liver carcinogenesis. But, it clearly demonstrated that the role of PPARα was dominant and that liver cancer estimates based upon PPARα (+/+) rodents would be dramatically overestimated if it were assumed that genotoxicity contributed significantly.

Liver toxicity, particularly cytotoxicity and reparative hyperplasia play a critical role in the liver carcinogenicity of chloroform in mice (Butterworth *et al.*, 1995). A similar role has been suggested for TCE (Bogen and Gold, 1997). While TCE does induce some liver toxicity it tends to be minimal (Barton and Das, 1996). Elevations in serum enzyme levels indicative of liver damage tend to be small by comparison with liver toxicants such as 1,1-dichloroethylene (Andersen and Jenkins, 1977; Buben and O'Flaherty, 1985). Liver pathology is not severely necrotic even at high doses, but rather shows limited necrosis and significant centrilobular changes associated with peroxisome proliferation (Elcombe *et al.*, 1985). Therefore, cytotoxicity does not appear likely to be the dominant factor leading to liver carcinogenesis.

There are significant differences between TCE and PERC, notably severe fatty infiltration with PERC (Buben and O'Flaherty, 1985; Odum *et al.*, 1988). Both PPARα and PPARγ are involved in regulating lipid metabolism (Schoonjans *et al.*, 1996; Peters *et al.*, 1997a), but it appears unlikely that TCA would be a ligand for PPARγ because these receptor isoforms largely respond to different ligands. Fatty liver was not observed with TCA, TCE, or pharmaceutical PPARα-ligands. So, it is likely due to the highly lipophilic parent compound, PERC, and is not PPARα mediated.

A strong case can be made for a PPARα-mediated mode of action driving the observed liver cancer incidence in mice exposed to TCE and TCA, while other modes of action must be considered highly speculative or potential minor contributors. Therefore, it is appropriate to

evaluate the dose response for liver cancer for TCE and TCA based upon a PPARα-mediated mode of action. As will be analyzed below, the quantitative response following PERC exposure suggest that the fatty liver pathology contributes to the cancer response altering the dose response in comparison with TCA.

PPARα-MEDIATED MODE OF ACTION FOR LIVER CARCINOGENESIS

The overall process by which PPARα-ligands induce liver carcinogenesis has become increasingly clear since the cloning of PPARα and demonstration that it was essential to the carcinogenicity of WY-14,643 (Peters *et al.*, 1997b; Gonzalez *et al.*, 1998). PPARα activation alters a wide range of cellular responses, so it is said to induce a pleiotropic response (Reddy and Chu, 1996). These responses are induced by endogenous (*i.e.* specific lipids and cytokines) or exogenous ligands for the receptor (Gottlicher *et al.*, 1992; Krey *et al.*, 1997; Lin *et al.*, 1999). The responses are PPARα-specific as indicated by the lack of responses in genetic knock out mice, which still express other PPAR subtypes (Peters *et al.*, 1997a; Peters *et al.*, 1997b; Gonzalez *et al.*, 1998; Peters *et al.*, 1998).

The degree and nature of responses vary with the dose, duration of exposure, and identity of the exogenous ligand (Barrass *et al.*, 1993; Belury *et al.*, 1998). These variations with different PPARα-ligands are likely due to some combination of their different binding affinities and the induced conformations of the receptor-ligand complexes. Conformational changes induced by ligand binding have not been studied with PPARα, but they play critical roles in gene activation for other members of this nuclear hormone receptor superfamily (Wagner *et al.*, 1995; Brzozowski *et al.*, 1997; Shibata *et al.*, 1997; Shiau *et al.*, 1998) Variations in the conformation of the receptor-ligand complex are important for the affinity of binding to DNA response elements and accessory proteins, *i.e.* the coactivators, corepressors, and general transcription factors, that are essential to the cell-specific alterations in gene expression (O'Malley *et al.*, 1995; Katzenellenbogen *et al.*, 1996; Shibata *et al.*, 1997).

One interesting question is why TCA induces liver tumors in mice and not rats, while many PPARα-ligands are effective in both species (Ashby *et al.*, 1994; DeAngelo *et al.*, 1997). There

is a weak response to TCA in rats consisting of peroxisome proliferation and some liver enlargement (Elcombe, 1985; DeAngelo *et al.*, 1989; Mather *et al.*, 1990; Berman *et al.*, 1995). Thus, liver tumors are not observed in rats despite the facts that they are a PPARα-responsive species and TCA is a ligand capable of inducing the full range of pleiotropic responses in mice. This suggests that the TCA-PPARα complex in rats is not able to fully elicit responses due to some combination of lower affinity and/or altered conformation as compared to that complex in mice (Keller *et al.*, 1997).

The dominant hypothesis for the mode of action leading to cancer is that PPARα-is activated by exogenous ligands causing alterations in cell cycling that promote altered cells, such as the spontaneously occurring basophilic foci (Kraupp-Grasl *et al.*, 1990; Kraupp-Grasl *et al.*, 1991; Roberts, 1996; Roberts *et al.*, 1997). That cell cycling alterations overlaid on the normal background of physiological processes can lead to cancer is supported by the finding of mammary tumors in mice genetically engineered to overexpress cyclin D1, a key regulatory protein in cell cycling (Wang *et al.*, 1994). Alterations in oxidative stress have also been suggested to play a role or to serve as a good indicator of potential carcinogenicity (Ashby *et al.*, 1994; Rao and Reddy, 1996). Mixed results have been obtained for the role of oxidative changes, for example, overexpression of acyl-CoA oxidase transformed mammalian cells so they were tumorigenic in nude mice (Chu *et al.*, 1995), yet mice that were genetically engineered to lack expression of the same enzyme developed cancer (Fan *et al.*, 1998). Measures of oxidative damage following exposure to PPARα-ligands have been positive in some studies (Channel *et al.*, 1998) and negative in others (Soliman *et al.*, 1997). Alterations in cell cycling and peroxisomal proliferation induced by prototypical PPARα-ligands have an absolute requirement for PPARα as indicated by the studies in knock out mice (Peters *et al.*, 1997a; Peters *et al.*, 1997b; Aoyama *et al.*, 1998; Peters *et al.*, 1998). Efforts continue, however, to sort out the exact roles and relative importance of the various pleiotropic responses mediated by PPARα in the development of cancer (Gonzalez *et al.*, 1998).

PPARα-ligands induce mitogenic effects in rodent livers by altering expression of cytokines (*e.g.* TNFα), which induce alterations in cyclins and cyclin-dependent kinases in hepatocytes through several signaling cascades (Bojes and Thurman, 1996; Rininger *et al.*, 1996; Bojes *et al.*,

1997; Grasl-Kraupp *et al.*, 1997; Rokos and Ledwith, 1997; Rose *et al.*, 1997; Rose *et al.*, 1999). The response of the liver is to attempt to inhibit cell proliferation (Rumsby *et al.*, 1994). For most PPARα-ligands, including TCE and TCA, the inhibitory response shuts down the initial cell proliferation causing the characteristic proliferative burst, although a few ligands are able to sustain cell proliferative activity resulting in greater potency and more rapid development of liver cancer (Marsman *et al.*, 1988; Channel *et al.*, 1998). Thus, the liver has two simultaneous competing signals, proliferative and antiproliferative, as long as exposure to the PPARα-ligand continues. Under these conditions, the potential responses are limited: 1) remove the signaling ligand, 2) continue responding to the antiproliferative signal, 3) undergo apoptosis, or 4) transform to a phenotype that can escape the inhibitory pathways and continue responding to the proliferative signals (Andersen *et al.*, 1995; Rininger *et al.*, 1996).

In addition to stimulating mitogenesis, PPARα-ligands can inhibit apoptosis thus preventing cell loss as well as stimulating cell division (Rumsby *et al.*, 1994; Roberts *et al.*, 1995; Roberts, 1996; Grasl-Kraupp *et al.*, 1997; Schulte-Hermann *et al.*, 1997; Christensen *et al.*, 1998; Gill *et al.*, 1998a; Gill *et al.*, 1998b; Roberts *et al.*, 1998). The inhibition by PPARα-ligands occurred after apoptosis was induced experimentally by different mechanisms including exposure to transforming growth factor-β (TGF-β) or reexposing animals to PPARα-ligands after discontinuing dosing with the PPARα-ligands. Thus, selective conditions are established in the liver that would give a growth advantage to cells that transform to an inhibition-nonresponsive phenotype. Cells with this characteristic have been shown to arise spontaneously in an age dependent-manner and to have distinct characteristics from most other hepatocytes or spontaneous proliferative lesions (Kraupp-Grasl *et al.*, 1990; Cattley *et al.*, 1991; Kraupp-Grasl *et al.*, 1991). It has also been hypothesized that oxidative stresses arising from the induction of peroxisomes could induce DNA alterations contributing to the transformation of hepatocytes (Rao and Reddy, 1996).

Thus, while details of the mechanism of action for induction of liver cancers by PPARα-ligands remain to be elucidated, the mode of action or general character of the process is clear. These ligands induce pleiotropic responses in liver cells including alterations in cell cycling. In response to mitogenic stimuli from the PPARα-ligands, the liver mounts a mitoinhibitory

response creating an environment that will selectively provide a growth advantage to cells that escape this inhibitory response (Rininger *et al.*, 1996). These cells give rise to foci, which over time with continued exposure become cancerous lesions.

PRECURSORS TO LIVER CANCER USEFUL FOR DOSE-RESPONSE ANALYSIS

Cancer studies often use relatively few dose groups and high doses in order to maximize the potential to observe responses. Therefore, if the mode of action for a chemical-induced cancer response has been identified, it may be possible to use precursor events early in the cancer process to better define the dose-response expected for the development of cancer (EPA, 1996).

Oral gavage cancer studies with mice exposed to TCE have used high doses of approximately 1000 and 2000 mg/kg/day (NCI, 1976; NTP, 1983), while studies with PERC used approximately 400/500 and 800/1100 mg/kg/day in females and males, respectively (ATSDR, 1997a). Drinking water studies with mice exposed to TCA have used wider ranges of concentrations resulting in exposures between approximately 7 and 600 mg/kg/day (Bull *et al.*, 1990; DeAngelo and Daniel, 1990; Pereira, 1996). Inhalation studies with mice exposed to TCE used concentrations between 100 and 600 ppm while studies with PERC used 100 and 200 ppm (ATSDR, 1997a). Due to it's low volatility, there are no inhalation studies with TCA. By contrast, shorter duration studies of liver effects with TCE have used doses ranging between 18 and 3200 mg/kg/day for 1.5 or 6 months (Tucker *et al.*, 1982; Buben and O'Flaherty, 1985) or 37 and 300 ppm for 30 days (Kjellstrand *et al.*, 1983). Thus, these studies used much wider ranges of concentrations than the cancer studies and would better describe the dose-response relationship, provided appropriate endpoints were measured.

There are several possibilities that might be considered as precursor events for use in dose-response analysis for liver carcinogenicity of PPARα-ligands. These include a sensitive measure of receptor activation such as induction of CYP4A, an alteration in a specific liver activity such as increased cell proliferation or peroxisome proliferation, an integrated measure of PPARα-mediated liver effects such as increased liver to body weight ratio (LW/BW), or preneoplastic foci. No data are available for this last endpoint with TCE- or PERC-exposed animals, so it was

not considered further although limited data from TCA-exposed mice exists (Bull *et al.*, 1990; Pereira, 1996).

CYP4A is induced early after exposure to PPARα, so it is a potential candidate effect for precursor dose-response analysis. Induction of CYP4A in rodents is both temporally and mechanistically related to peroxisome proliferation. Inhibition of CYP4A activity following exposure to PPARα-ligands, such as clofibrate, inhibits the increase in peroxisomal β-oxidation (Milton *et al.*, 1990; Bell and Elcombe, 1991; Gibson, 1992; Kaikaus *et al.*, 1993). This suggests that the induction of CYP4A results in the metabolism of endogenous substrates to PPARα-ligands (or higher affinity ligands), facilitating activation of genes for the broader range of responses involved in peroxisome proliferation (Kaikaus *et al.*, 1993; Bocos *et al.*, 1995). It has not been determined if CYP4A activity is a requirement for PPARα-mediated alterations in cell cycling, so the extent to which CYP4A induction is causally related to liver carcinogenesis is unclear. The dose-response characteristics for induction of CYP4A have been compared with those for a few other effects (Bars *et al.*, 1993; Belury *et al.*, 1998). CYP4A induction is a sensitive response; induction of β-oxidation occurs at higher doses within an order of magnitude of the CYP4A-inducing dose (Belury *et al.*, 1998). Based upon available information, CYP4A induction could be considered an appropriate precursor event for use in dose-response assessment for PPARα-mediated liver carcinogenesis. If this were done, the regional induction of CYP4A in liver (Bell and Elcombe, 1991; Bell *et al.*, 1991; Bell *et al.*, 1992) would need to considered. This has been shown to be critical to interpreting the dose-response for other nongenotoxic inducers of cytochromes P450 (Andersen *et al.*, 1997a; Andersen *et al.*, 1997b; Andersen and Conolly, 1998). For TCE and PERC, induction of CYP4A is not a feasible precursor event due to the absence of data at doses much below those at which cancer was observed.

Measures of cell proliferation or altered expression of genes involved in altered cell cycling would be a second potential precursor endpoint. The dose-response characteristics for expression of *c-myc* (Belury *et al.*, 1998) and BrdU labeling (Budroe *et al.*, 1992) have been compared with other endpoints such as β-oxidation or increased LW/BW. There are mouse strain-dependent differences in the relative dose-dependency of these responses, but overall they

appear to occur within an order of magnitude of each other. One of the difficult issues about using cell proliferation as a precursor event is that it is generally a transient effect, except with a few potent PPARα-ligands, such as WY14,643, so the timing of measuring cell proliferation would be critical (Budroe *et al.*, 1992). Again, there are insufficient data for TCE or PERC to make use of this endpoint, although it might be appropriate for PPARα-ligands with more complete data.

Peroxisomal proliferation or increases in β-oxidative activity have been well characterized following responses to TCE, PERC, and other PPARα-ligands. There is an 80% correlation of peroxisomal proliferation and hepatocarcinogenic activity of PPARα-ligands (Ashby *et al.*, 1994). However, there continues to be great controversy over whether peroxisomal proliferation is a necessary precursor for cancer or a response that is highly correlated because it is also PPARα-mediated (Ashby *et al.*, 1994; Citron, 1995). Therefore, other options for dose metrics were considered preferable.

Another option was to use increased LW/BW as a precursor event. Altered LW/BW is an attractive option because:

1. It is an integrated indicator of cell proliferation, peroxisome proliferation, and the pleiotropic responses of the liver to PPARα-ligands, so it avoids concerns about selecting changes in expression of a single gene that may not reflect the overall cancer process.
2. It is mechanistically relevant because it reflects the changes in liver function that create the selective environment believed critical to the carcinogenicity of PPARα-ligands, *i.e.* mitogenicity followed by suppression of proliferation.
3. Like the liver cancer response, increased LW/BW is reversible upon cessation of dosing.
4. This is a response to PPARα-ligands that is frequently observed, so it is broadly applicable.
5. Increased LW/BW is temporally an early precursor event relative to carcinogenesis that achieves a maximal response with constant dosing in a matter of weeks.

6. This is a response that occurs at approximately the same dose as other PPARα-mediated endpoints that are mechanistically relevant, *e.g.* increases in mitogenesis, inhibition of apoptosis, and β-oxidation. In C57Bl/6N mice, mitogenicity occurred at a 3-fold higher dose, and with BALB/c mice it was weakly evident at a dose 10-fold lower than that at which increased LW/BW was statistically significant with clofibrate (Budroe *et al.*, 1992). With TCE little or no increase in cell proliferation was observed at 400 mg/kg/day in B6C3F1 mice, a dose 4-fold greater than that giving a statistically significant increase in LW/BW (Buben and O'Flaherty, 1985; Channel *et al.*, 1998).

There are good data for TCE-induced increases in LW/BW at a range of doses beginning significantly lower than the dose at which cancer was observed (e.g., Buben and O'Flaherty, 1985). Thus, increased LW/BW is a mechanistically and temporally relevant endpoint that is adequately sensitive. The data on this endpoint describe the dose-dependency of liver response down towards the more relevant low dose region.

A limitation of using alterations in LW/BW may be some lack of specificity for PPARα-mediated events. For example, the large accumulation of lipid with PERC exposure, which is not observed in TCE-exposed animals, appears to contribute to the magnitude of the response (see analysis of TCA dose metrics below) (Buben and O'Flaherty, 1985; Odum *et al.*, 1988). Thus, for PERC, LW/BW appears to result from some combination of different modes-of-action dependent upon the parent compound and the major metabolite, TCA. There are minor metabolites of PERC that might also be involved, but this is an unstudied area.

Two studies, one using corn oil gavage of TCE (Buben and O'Flaherty, 1985) and the other inhalation (Kjellstrand *et al.*, 1983), were selected as the studies with the widest dose range and longest exposure durations, although there are a large number of studies that evaluated this endpoint (Barton and Das, 1996; Barton and Clewell, 1998). The inhalation studies demonstrated that the increased LW/BW occurs over the first 30 days and remains constant at 90 days of exposure (Kjellstrand *et al.*, 1981; Kjellstrand *et al.*, 1983).

TCE, PERC AND TCA PHARMACOKINETICS

Physiologically-based pharmacokinetic models for TCE and PERC were used to estimate tissue dose metrics for TCA formed by metabolism (Gearhart *et al.*, 1993; Clewell *et al.*, 1994; Clewell *et al.*, 1999). The PBPK model for TCE was modified to simulate oral dosing (*i.e.* gavage or drinking water) with TCA.

Several dose metrics were calculated using the models. These included peak concentrations in blood of TCE, PERC, or TCA and the average daily AUC for TCE, PERC, or TCA. The average daily AUC is generally used directly for noncancer endpoints, but for cancer effects a lifetime average daily dose (LADD) is typically calculated from the average daily AUC by adjusting for less than lifetime exposures. These dose metrics estimated with the pharmacokinetic models are reported in subsequent tables.

The model parameters for simulating TCE exposures resulting in the formation of TCA are presented in Table 2. These values are those developed by Clewell *et al.* (1999) with modifications as described here. The absorption of TCE given by oral gavage to mice was simulated with a first order rate constant (KAS) of 0.3 hr^{-1} to more closely match the total metabolism observed by Prout *et al.* (1985). Further, it was assumed no DCA was produced as recent studies indicate previous data were unreliable and DCA formed in TCE-exposed mice was below the quantitation limit (Ketcha *et al.*, 1996; Merdink *et al.*, 1998; Barton *et al.*, 1999).

Studies of the noncancer and cancer effects following TCA exposure in mice all used drinking water as the exposure vehicle (Table 1). No pharmacokinetic data were available from drinking water studies. Limited information was available for aqueous gavage (Larson and Bull, 1992a) and *i.p.* injection (Fisher *et al.*, 1991). The PBPK model for TCE was modified to simulate dosing with TCA by oral gavage and drinking water. The critical elements of the new equation for the rate of change of TCA in a volume of distribution (RATCA in mg/h) are:

$$RATCA = RAO*TCETCA + KTZER - KUT * ATCA$$

Oral gavage dosing is described by the rate of oral absorption (RAO) from a two-compartment gastrointestinal tract model previously described in Staats et al. (1991) and Clewell et al. (1999), absorption from drinking water (KTZER), and first-order urinary elimination (KUT). This equation is integrated to obtain the amount of TCA in the volume of distribution (ATCA). The rate of entry of TCA into the volume of distribution from drinking water was assumed to be constant over a 24-hour period; thus KTZER was calculated from the average daily dose (mg/kg/day) by multiplying by body weight and dividing by 24 hours/day. The oral gavage dose (PDOSE) in the model was allowed to be either TCE or TCA through use of a parameter (TCETCA) that set RAO to zero either in the TCE model (*i.e.* when dosing with TCA) or in the TCA model (*i.e.*, when dosing with TCE). In the equation illustrated, TCETCA would equal 1.0 for dosing with TCA, so RAO would have a non-zero value, while it would equal 0.0 if dosing with TCE. Other terms in the equation for RATCA reflecting production of TCA from other metabolites of TCE have not been described here because they are not relevant to TCA dosing and have previously been described for modeling TCE pharmacokinetics (Clewell *et al.*, 1999).

The PERC model was that previously described by Gearhart *et al.* (1993). Parameter values are presented in Table 3. This model is similar to many other published PBPK models except it includes two fat compartments, one being more poorly perfused than the other. The model also has a compartmental description (i.e. volume of distribution and clearance) for TCA formed from metabolism of PERC.

Simulation of TCE and PERC pharmacokinetics using these models has been described extensively elsewheres, so it will not be repeated here (Gearhart *et al.*, 1993; Clewell *et al.*, 1994; Clewell *et al.*, 1999).

Modeling of TCA pharmacokinetics in mice has not received extensive attention nor is there substantial pharmacokinetic literature upon which to base parameter estimates for such modeling. A volume of distribution for TCA in mice of 0.236 was reported following intraperitoneal dosing (Fisher *et al.*, 1991). Blood time courses following oral gavage dosing with 20 and 100 mg/kg TCA in water have also been reported (Larson and Bull, 1992a). These data are not compatible with a single set of parameter values for volume of distribution, urinary

TABLE 2: PARAMETER VALUES USED IN THE PBPK MODEL FOR TCE AND TCA

Parameter	Abbrev.	Units	Mouse	Human
Body Weight	BW	kg	0.035* (0.02-0.035)	70
Alveolar ventilation	QPC	L/hr [a]	30	24* (18)
Cardiac Output	QCC	L/hr [a]	18	16.5* (13)
FRACTIONAL BLOOD FLOWS TO TISSUES:				
All Rapidly Perfused	QRC		0.594	0.699
Gut	QGC		0.141	0.181
Liver	QLC		0.02	0.046
Tracheo-Brochial	QTBC		0.005	0.025
All Slowly Perfused	QSC		0.406	0.301
Fat	QFC		0.07	0.052
FRACTIONAL VOLUMES OF TISSUES				
All Rapidly Perfused	VRC		0.165	0.101
Gut	VGC		0.042	0.017
Kidney	VKC		0.017	0.004
Liver	VLC		0.057	0.026
Tracheo-Bronchial	VTBC		0.0007	0.0007
All Slowly Perfused	VSC		0.638	0.651
Fat	VFC		0.072	0.214
PARTITION COEFFICIENTS				
Blood/Air	PB		14	9.2
Fat/Blood	PF		36	73
Gut/Blood	PG		1.8	6.8
Liver/Blood	PL		1.8	6.8
Rich/Blood	PR		1.8	6.8
Slow/Blood	PS		0.75	2.3
TB/Blood	PTB		1.8	6.8

[a]Scaled by body weight to the ¾ power

*Default value used for calculation of dose-response analysis dose metrics -- different values (shown in parentheses) were used for comparison with pharmacokinetic studies.

TABLE 2 (cont.)

Parameter	Abbrev.	Units	Mouse	Human
ORAL UPTAKE OF TCE				
Stomach to liver	KAS	/hr	0	0
Duodenum to liver	KAD	/hr	1* (0.27-1.1)	1
Stomach to duodenum	KTSD	/hr	10	10
Fecal excretion	KTD	/hr	0	0
TCE METABOLISM: OXIDATIVE PATHWAY				
Capacity	VMC	mg/hr [a]	39* (39-60)	10* (6-10)
Affinity	KM	mg/L	0.25	1.5* (1.5-3)
Fraction TCA	PO		0.035* (0.035-0.1)	0.08
TCOH OXIDATION TO TCA				
Capacity	VMOC	mg/hr [a]	1* (0.5-1.5)	25 (15-25)
Affinity	KMO	mg/L	0.25	250
TCOH GLUCURONIDATION				
Capacity	VMGC	mg/hr [a]	100	5
Affinity	KMG	mg/L	25	25
KINETICS OF GLUCURONIDE				
Biliary excretion	KEHBC	/hr [b]	0	0
Reabsorption	KEHRC	/hr [b]	0	0
Urinary excretion	KUGC	/hr [b]	0.5	3
VOLUME OF DISTRIBUTION and URINARY ELIMINATION				
TCA	VDTCAC	Fraction of BW	0.236** (0.4 - 0.6)	0.1
Urinary elimination of TCA	KUTC	/hr	0.035** (0.06-0.07)	0.023
TCOH	VDBWC	Fraction of BW	0.65	0.65
TCA ORAL UPTAKE				
Stomach to liver	KAS	/hr	0.0, 5.0	0
Duodenum to liver	KAD	/hr	0.0, 1.0	1
Stomach to duodenum	KTSD	/hr	3.0	10
Fecal excretion	KTD	/hr	0	0

[b]Scaled by body weight to the -¼ power

**TCA values shown were used for modeling TCA pharmacokinetics when produced from TCE. Values in parenthesis were used for simulating TCA pharmacokinetic studies and for modeling TCA in the cancer and LW/BW studies as described in the text.

clearance, and gastrointestinal absorption. This is apparent by inspection, since the peak concentrations are only about 3-fold different despite a 5-fold difference in the doses. The basis for the apparent dose-dependence of the kinetics is unclear. The 100 mg/kg dose could be simulated with a volume of distribution (VDTCAC) of 0.6, a urinary elimination rate (KUTC) of 0.06, an absorption rate from the first gastrointestinal compartment (KAS) of 5.0, a transfer rate from the first to the second compartment (KTSD) of 3.0, and an absorption rate from the second gastrointestinal compartment of 1.0, assuming no metabolism of TCA. The simulated AUCTCA for this dose was 1171 mg*h/L. The 20 mg/kg dose could be simulated with the following parameters: VDTCAC = 0.4, KUTC = 0.07, KAS = 5.0, KTSD = 0.0. An AUCTCA of 306 mg*h/L was obtained from the simulation which somewhat overestimated concentrations at early times.

The parameter values contrast with the values used for simulating TCA pharmacokinetics following production from TCE and PERC: VDTCA = 0.238, KUTC = 0.035. These parameter values would result in substantial overestimates of the reported blood concentrations following aqueous oral gavage, increasing the AUCTCA values by 2.5- to 4-fold. Conversely, the parameter values used to fit the Larson and Bull (1992) oral TCA exposure data result in a substantial underestimate of the blood concentrations following TCE and PERC exposures (Prout *et al.*, 1985; Gearhart *et al.*, 1993; Templin *et al.*, 1993)

To model the TCA drinking water studies, estimates of the daily dose of chemicals received by the animals were required. These values were obtained from the various papers (Table 4). Measurements of drinking water consumption by DeAngelo *et al.* (1989) found a decrease at the highest TCA concentration (31 mM or 5 g/L). The estimates of water consumption used in the other papers vary from 140 to 190 ml/kg.

TABLE 3: PARAMETER VALUES USED IN THE PBPK MODEL FOR PERC

Parameter	Abbrev.	Units	Mouse
Body Weight	BW	kg	0.035* (0.02-0.035)
Alveolar ventilation	QPC	L/hr [a]	20
Cardiac Output	QCC	L/hr [a]	15
FRACTIONAL BLOOD FLOWS TO TISSUES:			
All Rapidly Perfused	QRC		0.51
Liver	QLC		0.25
All Slowly Perfused	QSC		0.19
Fat 1	QFC1		0.03
Fat 2	QFC2		0.02
FRACTIONAL VOLUMES OF TISSUES			
All Rapidly Perfused	VRC		0.05
Liver	VLC		0.04
All Slowly Perfused	VSC		0.72
Fat 1	VFC1		0.08
Fat 2	VFC2		0.02
PARTITION COEFFICIENTS			
Blood/Air	PB		20
Fat/Blood	PF		75
Liver/Blood	PL		2.4
Rich/Blood	PR		2.2
Slow/Blood	PS		3.3
ORAL UPTAKE OF PERC			
Stomach to liver	KAS	/hr	0
Duodenum to liver	KAD	/hr	0.5
Stomach to duodenum	KTSD	/hr	0.5
Fecal excretion	KTD	/hr	0
PERC METABOLISM			
Capacity	VMAXC	mg/hr [a]	0.2
Affinity	KM	mg/L	2.0
First Order Metabolism	KFC	/hr	2.0
Fraction TCA	FTCA		0.9
VOLUME OF DISTRIBUTION			
TCA	VDC	fraction of BW	0.236
TCA URINARY ELIMINATION			
Elimination rate	KUC	/hr	0.035

[a]Scaled by body weight to the ¾ power.
[b]Scaled by body weight to the -¼ power

TABLE 4: TCA DRINKING WATER STUDIES: WATER CONCENTRATIONS, AVERAGE DAILY DOSE AND WATER CONSUMPTION

	Administered Concentration	Average Daily Dose (mg/kg/day)	Water Consumption (ml/kg)
CANCER			
Bull, 1990	1 g/L	168	168
	2 g/L	336	168
Herren-Freund *et al.*, 1987	5 g/L	950	190
Pereira, 1996	2 mmol/L (0.33 g/L)	47*	142
	6.67 mmol/L (1.1 g/L)	157	143
	20 mmol/L (3.3 g/L)	470	142
NONCANCER			
Parrish *et al.*, 1996	0.1 g/L	16.8*	168
	0.5 g/L	84	168
	2 g/L	336	168
DeAngelo *et al.*, 1989	6 mM (1 g/L)	131	131.0
	12 mM (2 g/L)	261	130.5
	31 mM (5 g/L)	442	88.4

*These doses were modeled using the parameters for TCA volume of distribution and urinary elimination based upon the 20 mg/kg aqueous gavage pharmacokinetic study. All others used the parameters based upon the 100 mg/kg dose in that study.

OBTAINING THE POINT OF DEPARTURE

Dose-response analyses used the cancer data and LW/BW as a precursor event in order to compare the results both in the observable range and for evaluating the appropriate MOE. Maximum likelihood estimates (ED10) and lower bounds (LED10) on the effective dose giving 10% response are reported. The LED10 is often considered an appropriate conservative estimate for the point of departure for dose-response analysis. The ED10 estimates are more appropriate for evaluating mechanistic issues because they are the best fit to the data.

To conduct the dose-response analysis, two modeling programs were used; the THRESH benchmark programs for quantal data, and the BENCH_C benchmark program for continuous

data (KS Crump Group, Ruston, LA). Multiple models were considered, with the one providing the best fit to the data selected as the model for the basis of the ED10 and LED10. For the quantal endpoints, the Polynomial and the Weibull models were considered. For the continuous data, the Linear, K-Power, Weibull, and Log-Logistic models were run, with the background response probability (P0) fixed at 0.05.

The many cancer studies with TCE, TCA, and PERC were carried out over a 20-year period by many different laboratories, so there was a great deal of variation in their exposure regimens and pathological analysis or reporting. Therefore, comparisons must be made with care to consider the impact of these variations. Notable differences include the duration of exposure, the time until sacrifice of animals, and reporting of carcinomas or combined carcinomas and adenomas. Differences in duration of exposure and time until sacrifice are particularly important for cancer induced by PPARα-ligands, because these tumors can be slow to develop and regress substantially once exposure is discontinued (Cattley *et al.*, 1991; Grasl-Kraupp *et al.*, 1997).

TCE DOSE-RESPONSE FOR HEPATOCARCINOGENICITY

Male mice had a greater hepatocarcinogenic response than did the females in oral and inhalation studies using B6C3F1 and Swiss mice (Table 1). This is also reflected in lower ED10 estimates for males, regardless of the dose metric used (Tables 5 and 6). Comparisons of the ED10s for male or female mice using the Daily AUC or LADD show them to vary 3-fold or less across the NCI (1976), NTP (1990), and Bell *et al.* (1978) studies (comparing carcinomas and combined adenomas and carcinomas, separately). Given the differences in exposure regimens, durations, and pathological analyses, this is reasonable agreement.

The ED10s for the Maltoni *et al.* (1986) study are noticeably higher than most of the other ED10s. The low incidence of cancers in this study likely reflects the one-year exposure with autopsy at natural death of the animals (approximately another year). This time period was designed to allow tumors with long latency times to express themselves, but these indicate the short exposure and extended post-exposure period combined to produce fewer tumors and perhaps regression of tumors. This is consistent with the behavior of other PPARα ligands.

The mouse liver cancer data for TCE were all obtained at one or two high oral doses that gave substantial responses; a broader range of three inhalation concentrations were used (Table 1). Estimates of the ED10 and LED10 extrapolate down substantially from the oral doses tested. For example, the LADD AUCs for male mice ranged from 2183 to 2654 mg*hr/L at the gavage doses used of approximately 1000 - 2000 mg/kg/day (see Table 5) while the LED10s were about 10-fold lower, 156 - 246 mg*hr/L (see Table 7). By comparison, the LADD following inhalation exposure of mice ranged from 688 to 1748 mg*hr/L while the LED10s were 410 to 1142 mg*hr/L.

One approach for using the data from the three studies would be to average the LED10 values. Typically the US EPA would used combined adenomas and carcinomas, but that information is not available for the NCI (1976) study. Alternatively, studies could be analyzed individually.

TABLE 5: LIVER TUMOR DOSE METRICS FOR TCA

Species	Dose	Sex	Daily AUC	LADD (AUC) (a)	CMAX (b)
		TRICHLOROETHYLENE			
Mouse	2027 mg/kg (e)	M	3067	2654	218
	1507 mg/kg (e)	F	2836	2454	203
	1013 mg/kg (e)	M	2522	2183	182
	1000 mg/kg (f)	M,F	2512	2488	182
	753 mg/kg (e)	F	2285	1977	166
	600 ppm (c)	M,F	1748	1748	157
	600 ppm (d)	M,F	1984	1488	175
	300 ppm (c)	M,F	1322	1322	123
	300 ppm (d)	M,F	1513	1135	138
	100 ppm (c)	M,F	798	798	76
	100 ppm (d)	M,F	917	688	85
human	1 ppm (g)		303	303	13
	1 mg/L (h)		14	14	0.6

TABLE 5 (cont.)

Species	Dose	Sex	Daily AUC	LADD (AUC) (a)	CMAX (b)
		TRICHLOROACETIC ACID			
Mouse	4.5 g/L (i)	M	10273	10273	428
	5 g/L (j)	M	11414	6695	476
	20 mmol/L (k)	F	5647	4468	235
	20 mmol/L (l)	F	5647	2792	235
	2 g/L (m)	M, F	4037	2019	168
	6.67 mmol/L (k)	F	1888	1492	79
	1 g/L (m)	M, F	2018	1009	84
	6.67 mmol/L (l)	F	1886	933	79
	2 mmol/L (k)	F	726	574	30
	2 mmol/L (l)	F	726	359	30
		PERCHLOROETHYLENE			
Mouse	927 mg/kg (n)	M	3132	2710	238
	667 mg/kg (n)	F	2316	2004	176
	464 mg/kg (n)	M	1665	1441	126
	200 ppm (o)	M,F	1098	1098	89
	333 mg/kg (n)	F	1243	1076	93
	100 ppm (o)	M,F	601	601	49
human	1 ppm (g)		5.2	5.2	0.22
	1 mg/L (h)		0.57	0.57	0.024

(a) Lifetime average daily area under the plasma concentration curve (mg-hrs/L)
(b) Maximum concentration achieved during exposure (mg/L)
(c) Inhalation, 6 h/d, 5 d/w, 104w (Bell, 1978)
(d) Inhalation, 7 h/d, 5 d/w, 78/104 w (Maltoni *et al.*, 1986; Maltoni *et al.*, 1988)
(e) Oil gavage, 5 d/w, 78/90 w (NCI, 1976)
(f) Oil gavage, 5 d/w, 103/104 w (NTP, 1990)
(g) Inhalation - lifetime continuous
(h) Drinking water - lifetime continuous
(i) Drinking water, continuous for 104 w (Ferreira-Gonzalez *et al.*, 1995)
(j) Drinking water, continuous for 61 w (Herren-Freund *et al.*, 1987)
(k) Drinking water, continuous for 576 d (Pereira, 1996)
(l) Drinking water, continuous for 360 d (Pereira, 1996)
(m) Drinking water, continuous for 52 w (Bull *et al.*, 1990)
(n) Oil gavage, 5 d/w, 78/90 w (NCI, 1977)
(o) Inhalation, 6 h/d, 5 d/w, 103 w (NTP, 1986)

TABLE 6: ED10s FOR LIVER CANCER FOR TCE, TCA AND PERC

Study	Endpoint	Sex	External (mg/kg/day)		Daily AUC (mg*hr/L)		LADD (mg*hr/L)	
			ED10	LED10	ED10	LED10	ED10	LED10
		TRICHLOROETHYLENE - GAVAGE						
NCI, 1976	Hepatocellular carcinoma	M	137	106	892	284	774	246
		F	619	349	2372	1619	2050	1400
NTP, 1990	Hepatocellular carcinoma	M	123	67	337	234	334	232
		F	317	173	996	609	986	603
	Hepatocellular carcinoma & adenoma	M	86	45	226	158	224	156
		F	195	105	572	170	567	367
		TRICHLOROETHYLENE - INHALATION						
Bell, *et al.*, 1978	Hepatocellular carcinoma	M	179	122	887	429	778	410
	Hepatocellular adenoma & carcinoma	M	113	82	702	309	574	288
		F	614	355	1761	1382	1766	1354
Maltoni, *et al.*, 1986	Hepatomas	M (BT 305)	515	358	1865	1522	1399	1142
		M/F	789	597	2206	1981	1654	1486
		TRICHLOROACETIC ACID - DRINKING WATER						
Pereira, 1996	Hepatocellular carcinoma	F (306d)	219	116	5341	2909	2641	1436
		F (576d)	120	86	1918	1435	1517	1135
	Hepatocellular adenoma & carcinoma	F (576d)	95	57	1591	906	1258	717
Herren-Freund, *et al.*, 1987	Hepatocellular adenoma	M (427d)	191	85	3916	1742	2297	1021
	Hepatocellular carcinoma	M (427d)	180	87	3679	1779	2158	1044
		PERCHLOROETHYLENE - INHALATION						
NTP, 1986	Hepatocellular carcinoma	M	30	21	165	119	165	119
		F	64	36	430	249	430	249
	Hepatocellular adenoma & carcinoma	M	23	12	169	71	169	71
		F	57	28	369	197	369	179
		PERCHLOROETHYLENE - GAVAGE						
NCI, 1977	Hepatocellular carcinoma	M	73	52	341	248	295	214
		F	75	58	371	287	321	248

TCA DOSE-RESPONSE FOR HEPATOCARCINOGENICITY

Although several studies have all demonstrated that B6C3F1 mice develop hepatocellular carcinomas due to TCA exposure, there is no published lifetime study with multiple dose levels. Such a study, particularly in male mice, would be valuable for evaluating whether TCA is kinetically competent to induce cancers from TCE and PERC. Absent this more complete information, several useful comparisons can be made based upon the values for ED10 and LED10 (Table 6, 7).

Pereira (1996) reports a greater incidence of hepatocellular carcinomas following 576 days than 360 days exposure to TCA in drinking water (Table 1). The corresponding ED10 and LED10 are lower following the longer exposure (see Pereira entries in Table 6). That is, for shorter exposures, a higher internal dose of TCA is required to achieve the same (10%) cancer incidence. This study helps support the idea that the higher values for ED10 and LED10 in the Maltoni *et al.* (1986) reflect the shorter exposure (78 weeks) compared to the Bell *et al.* (1978) study (104 weeks). In the Pereira (1996) study, animals were exposed to TCA until they were sacrificed, so unlike the Maltoni *et al.* (1986) studies, it did not permit post-exposure recovery.

There is good consistency in the TCE and TCA studies with female mice for the values of the dose metrics for TCA estimated to give a 10% response (see Table 6). For example, the ED10 using the Daily AUC and LADD AUC for TCA from Pereira (1996) were 1918 and 1517 mg*hr/L for carcinomas in females following 576 day exposure. By comparison, these ED10s for TCA were 2372 and 2050 mg*hr/L for carcinomas in females in the NCI (1976), and 996 and 986 mg*hr/L for the NTP (1990) studies with TCE exposure. The similarity of the tissue dose of TCA associated with a 10% incidence of hepatocellular carcinomas indicates that the cancers observed with TCE can be accounted for by the TCA formed by its metabolism.

Unfortunately, the data for males are limited. The dose metrics associated with a 10% response in the 61 week TCA drinking water study by Herren-Freund *et al.* (1987) are substantially higher than the TCA dose metrics estimated for a 10% response in males following TCE exposure (*e.g.* LADD of 2158 versus 774 or 334 mg*hr/L for dosing with TCA versus TCE, see Table 6).

However, data presented in abstract form (DeAngelo and Daniel, 1990) for males dosed with TCA for 95 weeks found a 10% response at an LADD of 486 mg*hr/L, which is similar to the 784 and 334 mg*hr/L for 10% response following TCE dosing in the two corn oil gavage bioassays. Thus, the data again show that the cancer response with TCA is very dependent upon the duration of exposure and that TCA production from TCE appears sufficient to explain the observed TCE hepatocarcinogenicity.

TCE AND TCA DOSE-RESPONSE FOR LW/BW

The Buben and O'Flaherty (1985) study provides an extensive dose-response for LW/BW spanning the doses used in the cancer bioassay (Tables 7, 8). The daily AUCs for the ED10 and LED10 were 956 and 768 mg*hr/L, respectively, for TCA produced from metabolism of TCE (Table 9). These values are similar to, though higher than, the ED10 and LED10 values obtained from the two oral TCE liver cancer studies. More limited dose ranges are available for TCA, with ED10 and LED10 values of 543 and 348 mg*hr/L, respectively, obtained from mice treated for 71 days (Parrish *et al.*, 1996). These values are similar to, although lower than, those for TCA produced from TCE in the study by Buben and O'Flaherty (1985).

The Kjellstrand et al. (1983) study also provides a somewhat more extensive dose-response for LW/BW than was used in the cancer studies, though only the 37 ppm concentration is much below the lowest concentration (100 ppm) in the cancer studies (Tables 1, 7). The dose metrics at all concentrations of this inhalation study are notably higher than those in the oral gavage study (Table 8). Thus, the daily AUC at the ED10 for LW/BW increase was 956 mg*hr/L in the gavage study versus 2476 mg*hr/L in the continuous inhalation study.

As was described above in the mode of action section, it is the alterations in cell cycling (either or both proliferation and apoptosis) and perhaps peroxisomal proliferation that are critical to cancer induction. These two factors are responsible for the increased LW/BW with TCA and TCE. In the absence of this pleiotropic response, no cancer would be expected. Therefore, the mode of action and dose response information for LW/BW is useful for evaluating the dose response expected below the high cancer bioassay doses.

TABLE 7: SUMMARY OF CHANGES IN LW/BW FOLLOWING EXPOSURE TO TCE, TCA AND PERC

Study	Strain/ Species	Sex	Duration	Administered Dose [a]	Liver Weight/ Body Weight (%)[b]
			TRICHLOROETHYLENE — GAVAGE		
Buben and O'Flaherty, 1985	Swiss-Cox Mice	M	5 d/w, 6w	0	5.22±0.44
				100	5.84±0.45**
				200	5.99±0.45**
				400	6.51±0.42**
				800	7.12±0.42**
				1600	8.51±0.69**
				2400	8.82±0.52**
				3200	9.12±0.3**
			TRICHLOROETHYLENE — INHALATION		
Kjellstrand *et al.*, 1983	NMRI Mice	M	continuous, 30 days	0	3.82±0.61[c]
				37	4.39±0.21
				75	5.63±0.35
				150	7.11±0.84
				300	8.81±0.89
		F		0	3.46±0.49[c]
				37	3.63±0.22
				75	4.57±0.44
				150	6.16±0.46
				300	8.86±0.86
			TRICHLOROACETIC ACID — DRINKING WATER		
Parrish *et al.*, 1996	B6C3F1 mice	M	21d	0	5.4±0.24
				0.1 g/L	5.3±0.24
				0.5 g/L	6.1±0.49
				2.0 g/L	7.2±0.24**
		M	71d	0	5.1±0.24
				0.1 g/L	4.6±0.49**
				0.5 g/L	5.8±0.49**
				2.0 g/L	6.9±0.49**
DeAngelo *et al.*, 1989	B6C3F1 mice	M	14d	0	5.1±0.49
				131	5.5±0.49
				261	5.9±0.73
				442	7.1±0.98**

** Statistically significant increase compared to corresponding control value ($p<0.05$).

[a] Reported in units of mg/kg/day unless specified.

[b] Reported as mean ± standard deviation.

[c] Individual control groups reported in the paper were averaged for this table.

TABLE 7 (cont.)

Study	Strain/ Species	Sex	Duration	Administered Dose [(a)]	Liver Weight/ Body Weight (%)[(b)]
			PERCHLOROETHYLENE — GAVAGE		
Buben and O'Flaherty, 1985	Swiss-Cox mice	M	5 d/w, 6w	0	5.21±0.46
				20	5.51±0.40
				100	5.97±0.40**
				200	6.45±0.46**
				500	7.35±0.62**
				1000	7.89±0.70**
				1500	8.10±0.66**
				2000	9.00±0.27**

** statistically significant increase compared to corresponding control value ($p<0.05$).

[a] Reported in units of mg/kg/day unless specified.

[b] Reported as mean ± standard deviation.

TABLE 8: LIVER NONCANCER DOSE METRICS FOR TCA

Species	Dose	Daily AUC (mg*hr/L)	CMAX [(a)] (mg/L)
	TRICHLOROETHYLENE (Gavage)		
mouse**	3200 mg/kg (b)	3417	239
mouse**	2400 mg/kg (b)	3198	226
mouse**	1600 mg/kg (b)	2883	206
mouse**	800 mg/kg (b)	2334	169
mouse**	400 mg/kg (b)	1771	130
mouse**	200 mg/kg (b)	1263	96
mouse**	100 mg/kg (b)	897	71
	TRICHLOROETHYLENE (Inhalation)		
mouse**	300 ppm (c)	6414	267
mouse**	150 ppm (c)	4656	194
mouse**	75 ppm (c)	3598	150
mouse**	37 ppm (c)	2770	115
	TRICHLOROACETIC ACID		
mouse**	442 mg/kg (d)	5311	221
mouse**	2 g/L (e)	4037	168
mouse	261 mg/kg (d)	3136	131
mouse	131 mg/kg (d)	1574	66
mouse**	0.5 g/L (e)	1009	42
mouse**	0.1 g/L (e)	260	11

TABLE 8 (cont.)

Species	Dose	Daily AUC (mg*hr/L)	CMAX [(a)] (mg/L)
	PERCHLOROETHYLENE		
mouse**	2000 mg/kg (b)	6468	497
mouse**	1500 mg/kg (b)	4917	376
mouse**	1000 mg/kg (b)	3358	256
mouse**	500 mg/kg (b)	1777	134
mouse**	200 mg/kg (b)	792	59
mouse**	100 mg/kg (b)	438	33
mouse	20 mg/kg (b)	109	8

** Significantly increased liver weight/body weight ratio in at least one study.
(a) Maximum concentration achieved during exposure (mg/L)
(b) Oil gavage, 5 d/w, 6 w (Buben and O'Flaherty, 1985)
(c) Inhalation, 24 h/d 7d/2k (Kjellstrand et al., 1983)
(d) Drinking water, continuous for 14 days (DeAngelo, *et al.* 1989).
(e) Drinking water, continuous for 21 or 71 days (Parrish, *et al.* 1996).

TABLE 9: ED10s AND LED10s FOR INCREASES IN LW/BW FOR TCE, TCA AND PERC

Study		Sex	Daily AUC (mg*hr/L)	
			ED10	LED10
TRICHLOROETHYLENE -- GAVAGE				
Buben and O'Flaherty, 1985		M	956	768
TRICHLOROETHYLENE -- INHALATION				
Kjellstrand et al., 1983		M	2476	2173
		F	3107	2770
TRICHLOROACETATE—DRINKING WATER				
Parrish et al., 1996	21 d	M	661	457
	71 d	M	543	348
DeAngelo et al., 1989	14 d	M	2084	855
PERCHLOROETHYLENE -- GAVAGE				
Buben and O'Flaherty, 1985		M	86	42

PERC AND TCA DOSE-RESPONSE FOR CANCER AND LW/BW

Comparisons of the internal dose metrics for TCA associated with 10% response for cancer and LW/BW increase appear to present a different picture for animals dosed with PERC. The internal doses of TCA produced from PERC do not appear adequate to account for the 10% response (see Table 6 or 9) compared to internal doses of TCA associated with a 10% response following exposure to TCE or TCA. The difference is particularly large for the TCA dose metric (daily AUC) associated with the LED10 for increased LW/BW (Table 9). The dose-response analysis estimates a daily AUC ED10 for TCA of 86 mg*hr/L, while daily AUC ED10s of 500 to 700 mg*hr/L were estimated for TCA dosing of a similar duration. Comparisons of daily AUC or LADD associated with 10% cancer incidence similarly show these values are lower following PERC exposure than TCA exposure. As has previously been discussed, PERC causes a large increase in lipid accumulation in mouse liver, while small or no increases are seen following TCE and TCA exposures (Buben and O'Flaherty, 1985). Therefore, we have presented the hypothesis that this apparently non-PPARα mode of action of PERC may contribute to the tumor response. For PERC, the internal tissue dose of TCA does not appear adequate to fully explain the magnitude of the increased LW/BW or the hepatocarcinogenic response.

LOW DOSE EXTRAPOLATION

The PPARα-mediated mode of action drives the choice of the appropriate dose metric for evaluating the observed data, in this case the AUC for TCA, and therefore the pharmacokinetic modeling. Mode of action is also critical for selecting the appropriate low dose extrapolation method, and when a margin of exposure (MOE) approach is applied, the selection of the appropriate MOE. The remainder of the analysis focuses only on TCE due to the potential involvement of an additional mode of action with PERC, perhaps associated with parent compound and development of a fatty liver.

The PPARα-mediated mode of action leading to liver carcinogenesis requires significant alterations in cell cycling and possibly, increased β-oxidation and peroxisomal proliferation.

These events are widely considered to have thresholds or highly nonlinear dose-response relationships, although this is experimentally difficult to demonstrate (Fenner-Crisp, 1996). Biologically based analyses of related systems have identified two other characteristics of this system that also support the existence of highly nonlinear dose-response relationships.

Induction of several cytochromes P450 including CYP4A occurs regionally, specifically in the centrilobular region, with the induced area increasing as the dose of inducing compound is raised (Bell and Elcombe, 1991; Bell *et al.*, 1991; Bell *et al.*, 1992; Chen *et al.*, 1995). This indicates that cells switch from a "ligand-nonresponsive" (*i.e.*, uninduced state) to a "ligand-responsive" state as a function of ligand dose. Due to the slow metabolism of several of these inducing compounds, here TCA, the free or available concentration of the ligand is expected to be similar across the hepatic lobule. If anything, TCA would be higher in the periportal region because it would enter from the blood and be metabolized in the liver, so the regional response does not appear driven by tissue dosimetry, but rather by tissue response. Modeling indicated that a highly nonlinear dose-response was required to capture the phenomenon of regional induction (Andersen *et al.*, 1997a; Andersen *et al.*, 1997b; Andersen and Conolly, 1998).

The requirement for induction of CYP4A activity as a precursor for peroxisomal proliferation was previously discussed. Models for receptor-mediated gene activation found that induction of the enzyme required for synthesizing a high affinity ligand or the receptor could result in highly nonlinear dose-response relationships mimicking effects seen in life forms ranging from bacteria to mammals (Andersen and Barton, 1999). Induction of lauric acid 12-hydroxylase (ω-hydroxylase) activity by PPARα-ligands can produce a 5 - 10-fold increase in activity (Sharma *et al.*, 1988; Milton *et al.*, 1990). The modeling indicated this degree of induction could create a highly nonlinear dose-response. As previously mentioned, there is no data available that indicates whether CYP4A induction is required for PPARα-induced alterations in cell cycling as it is for induction of β-oxidation. Therefore, this is a plausible hypothesis for a biological factor creating a nonlinear dose-response relationship for hepatocarcinogenesis induced by PPARα-ligand, but additional experimental work would be required to determine if it is operable.

Concentrations of PPARα are also induced, but this occurs in response to increased glucocorticoid levels such as arise from fasting, stress, or diurnal variations (Lemberger *et al.*, 1994; Braissant *et al.*, 1996; Lemberger *et al.*, 1996; Plant *et al.*, 1998). Decreased food consumption due to reduced palatability can also produce conditions that increase PPARα-levels, presumably through the same mechanism as fasting (Sterchele *et al.*, 1996). This suggests PPARα increases observed in studies with PPARα-ligand exposed animals may be due to alterations in food consumption (Miller *et al.*, 1996). Autoinduction of a receptor (*i.e.* induction by it's own ligand) can create a highly nonlinear dose-response relationship (Andersen and Barton, 1999), but there is little evidence for autoinduction of PPARα (Gonzalez *et al.*, 1998). However, a nonlinear dose-response in liver responsiveness could also arise if PPARα-levels were dose-dependently induced through indirect mechanisms such as altered feeding. Alterations in food consumption resulting in PPARα induction would need to be accounted for in analyzing the dose response for the animal studies, but would be unlikely to be relevant for humans exposed to low doses.

Additional mechanistic studies, particularly ones examining dose-responses relationships between tissue concentrations and precursor events, would be valuable for developing biological-based models of PPARα-mediated liver responses and hepatocarcinogenesis. However, as described here, there are several factors that indicate hepatocarcinogenesis would drop off steeply below concentrations of PPARα-ligands inducing minimal alterations in liver function or LW/BW. Therefore, an MOE approach is justified for evaluating PPARα-induced liver carcinogenesis.

In a dose-response analysis for carcinogenic effects that are expected to have a nonlinear dose response, several factors are evaluated to establish the appropriate magnitude of the margin of exposure. These factors include the severity of the endpoint (*e.g.*, cancer or a precursor event) and the slope of its dose-response relationship, human variability, and interspecies extrapolation (EPA, 1996). In a biologically motivated analysis, both mode of action and pharmacokinetic considerations will assist in determining the need for specific uncertainty factors and how large they need to be. Mode of action considerations can be particularly important for evaluating

whether multiple uncertainty factors, which independently appear reasonable, are really addressing overlapping concerns.

ENDPOINT AND SLOPE

If the cancer data were used for the dose-response analysis, then a default factor of 10 would be applied. This factor addresses the severity of the endpoint. The default analysis compares the slope at the LED10 with the slope from the LED10 to the origin to determine if the default factor would be included. For trichloroethylene liver carcinogenesis, the slope is not sufficiently steep to differentiate it from linear, so the uncertainty factor of 10 would be applied.

Use of dose-response information for increased LW/BW has been proposed as mechanistically related to the development of cancer for PPARα-mediated liver carcinogenesis. It has been argued here that this effect is a measure of the PPARα-mediated pleiotropic response, particularly altered cell cycling, that is necessary for the carcinogenic process. Absent an increase in LW/BW, the alterations due to exposure to PPARα-ligands would be minimal or compensated for, such as that no cancer response would occur.

The ED10s and LED10s for hepatocellular carcinoma and increased LW/BW following gavage dosing show the relationship between the points of departure obtained with these two endpoints (Table 10 and Figure 1). Mechanistic comparisons are most appropriately based upon the ED10s; the LED10s are often preferred for developing health protective numbers because they incorporate estimates of uncertainty due to the quality of the quantitative data. The daily AUC for a minimal increase in LW/BW is similar to, though higher than, the internal dose of TCA associated with 10% cancer incidences in the gavage studies. There is a 2-fold difference in the maximum likelihood estimate of LADD between the NCI (1976) and NTP (1990) studies reflecting the higher incidence in the NTP (1990) study at a dose equivalent to the low dose in the NCI (1976) study (see Table 1). Use of combined adenomas and carcinomas would also decrease the TCA dose metric as is apparent in Table 10, for the NTP (1990) study or Bell *et al.* (1978).

Particularly notable is the impact of the more extensive oral dose-response data for LW/BW on the estimate of the lower bound, LED10. This value is much more similar to the maximum likelihood estimate than are the lower bounds for the cancer endpoints, which are based upon one or two dose groups in addition to the control groups. Thus, for oral dosing the point of departure based upon LW/BW is interpolated between measured data points, where those for cancer are extrapolated below the measured data. For inhalation, the daily AUC associated with the point of departure for LW/BW is much higher than that based upon cancer. The inhalation studies of LW/BW used continuous exposure, so they may not provide a reasonable comparison with the inhalation cancer studies.

TABLE 10: POINTS OF DEPARTURE ESTIMATED FOR LIVER CANCER AND INCREASED LW/BW

			ED10		LED10	
	Endpoint	Sex	Daily AUC	LADD	Daily AUC	LADD
NCI, 1976	Hepatocellular carcinoma	M		774		246
NTP, 1990	Hepatocellular carcinoma	M		334		232
	Hepatocellular carcinoma & adenoma	M		224		156
Buben and O'Flaherty, 1985	Increased LW/BW	M	956		768	
Bell *et al.*, 1978	Hepatocellular carcinoma	M		778		410
	Hepatocellular carcinoma & adenoma	M		574		288
Kjellstrand *et al.*, 1983	Increased LW/BW	M	2476		2173	

mg*hr/L

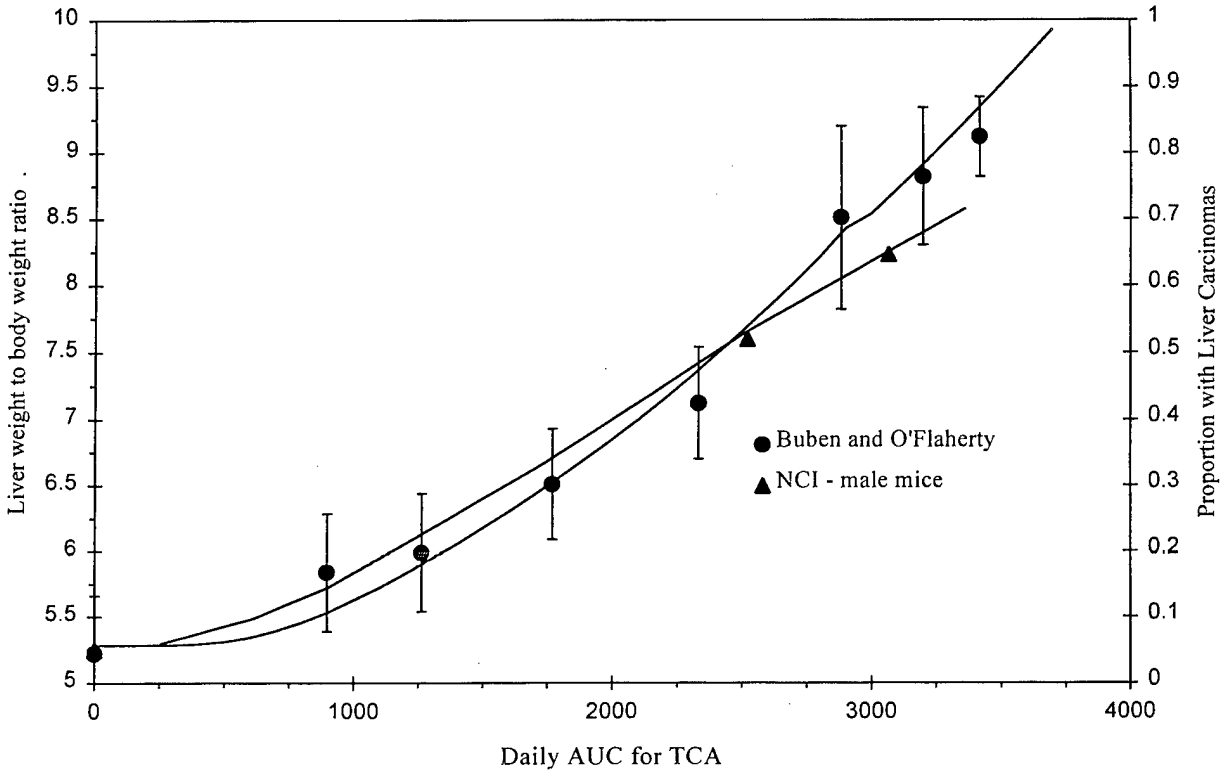


Figure 1: Comparison of Dose-Responses for Liver/Body Weight Ratio and Liver Tumors

HUMAN VARIABILITY

There is little known about the human variability with respect to PPARα expression or the responsiveness of regulated genes to ligand-receptor complexes (Perrone *et al.*, 1998). Therefore, a default factor of 10-fold would be used.

INTERSPECIES VARIABILITY

Use of PBPK modeling to obtain the human equivalent concentrations for the production of TCA from TCE and PERC accounts for the pharmacokinetic differences between species. Therefore, the remaining question concerns the relative sensitivity of humans and rodents for the

pharmacodynamic processes, *i.e.* the PPARα-mediated mode of action. A default uncertainty factor of 3 is often applied for the pharmacodynamic asset alone.

Studies of the effects of PPARα-ligands on livers from humans, monkeys, and other so-called nonresponsive species (*e.g.* guinea pig, hamster, dog) have failed to show the kinds of responses observed in rodents (Cattley *et al.*, 1998). The biological basis for this species-specificity of response continues to be studied. Humans have PPARα, but the levels appear to be about 10-fold lower than in rodents (Sher *et al.*, 1993; Mukherjee *et al.*, 1994; Auboeuf *et al.*, 1997; Palmer *et al.*, 1998). It should be noted that cloned PPARα expressed in human liver cells lines was active, suggesting that necessary accessory proteins, such as RXR, were not limiting *in vivo* responsiveness (Palmer *et al.*, 1998). If receptor concentrations were limiting, the low levels in humans could be sufficient to mediate the reduction in lipid levels, but not the more general pleiotropic responses observed in rodents; this remains under study (Gonzalez, 1997; Gonzalez *et al.*, 1998).

Most studies in nonresponsive species have focused upon measures of peroxisome proliferation; only human or monkey studies are reviewed here. Human studies included examinations of liver biopsies and limited epidemiological studies of patients treated with fibrate hypoglycemic drugs (reviewed in Ashby *et al.* 1994). No increase in LW/BW occurred in marmosets gavaged with ciprofibrate for 26 weeks or 3 years (Gibson, 1992; Graham *et al.*, 1994) or in other monkey species given PPARα-ligands (Short *et al.*, 1987; Kurata *et al.*, 1998). Small (2- or 3-fold) or no increases in β-oxidation were observed in marmosets or other monkeys as compared to large (*e.g.,* 10-fold) increases in rats (Holloway *et al.*, 1982a; Holloway *et al.*, 1982b; Gibson, 1992; Graham *et al.*, 1994). Little or no peroxisomal proliferation has been observed in monkeys treated with PPARα-ligands (Short *et al.*, 1987; Stott *et al.*, 1995; Kurata *et al.*, 1998). Small or no increases in β-oxidation or peroxisomal proliferation were seen with *in vitro* studies in primary monkey or human hepatocytes (Blaauboer *et al.*, 1990; Foxworthy *et al.*, 1990; Dirven *et al.*, 1993; Mennes *et al.*, 1994; Perrone *et al.*, 1998)

More recent studies have looked for *in vitro* alterations in CYP4A expression or changes in cell cycling. Little or no induction of CYP4A has been reported in monkey or human cells or tissue

slices (Dirven *et al.*, 1993; Lake *et al.*, 1996). Induction of cell proliferation by PPARα-ligands has not been observed with human hepatocytes (Parzefall *et al.*, 1991; James and Roberts, 1995); one study reported suppression of proliferation (Perrone *et al.*, 1998). No suppression of TGFβ-induced apoptosis was observed in human hepatocytes (Hasmall *et al.*, 1998; Perrone *et al.*, 1998); an increase or no effect was reported for basal rates of apoptosis.

These studies do not support the default assumption that humans are more sensitive than the rodent species used in the toxicity studies (*i.e.*, a factor of 3). Arguably, humans are much less sensitive and the interspecies factor for pharmacodynamics should be less than 1.0; some have argued humans are nonresponsive for the precursor events leading to PPARα-induced liver cancer. However, without additional mechanistic details about PPARα-mediated liver carcinogenesis, it may not be clear that PPARα-mediated carcinogenesis can be considered rodent-specific and therefore, irrelevant to humans (Citron, 1995). The intermediate position is taken here, therefore, that the liver cancer endpoint should be evaluated in the dose-response assessment of PPARα-ligands, but the interspecies extrapolation should assume at most equal sensitivity of humans and rodents (*i.e.*, a factor of 1.0).

LIVER CANCER DOSE RESPONSE ANALYSIS

The previous sections have outlined the components required in a biologically-based dose-response analysis for trichloroethylene induced liver cancer. The point of departure could be obtained by statistical curve fitting to the cancer data and extrapolation down to the lower bound on the dose associated with a 10% response. Alternatively, the point of departure could be the lower bound on a dose at which a minimal increase in LW/BW occurs. Absence of a substantial increase in LW/BW would indicate a minimal or absent pleiotropic response mediated by TCA as a PPARα-ligand thus protecting against the alterations in cell cycling required in the tumor promotion process. These points of departure (Table 10) obtained from the animal studies are expressed in the units (mg*hr/L) of the internal dose metric used in the analysis, AUCTCA. The human exposures that would result in this internal TCA dose metric would be estimated using a pharmacokinetic model parameterized for humans. Because the human model for TCE is virtually linear over a wide range of doses, a constant factor can be used to convert the internal

TABLE 11: CANCER DOSE RESPONSE ANALYSIS

Study	Endpoint	LED10 LADD or Daily AUC	MOE	Conversion to Human External Dose	Acceptable Exposure Limit	Allowable Concentration Drinking Water (μg/L)
ORAL					*(mg/kg/day)*	
NCI, 1976	Hepatocellular carcinoma	246	1000 100	479	0.0005 0.005	17.5 175
NTP, 1990	Hepatocellular carcinoma	232	1000 100	479	0.0005 0.005	17.5 175
	Hepatocellular carcinoma & adenoma	156	1000 100	479	0.0003 0.003	10.5 105
Buben and O'Flaherty, 1985	Increased LW/BW	768	100	479	0.016	560
					(ppm)	
Bell *et al.*, 1978	Hepatocellular carcinoma	410	1000 100	303	0.001 0.01	
	Hepatocellular carcinoma and adenoma	288	1000 100	303	0.001 0.01	
Kjellstrand *et al.*, 1983	Increased LW/BW	2173	100	303	0.07	

dose metric to the external human exposure. Conversion factors are required for the parameters defining each exposure scenario of interest (*i.e.*, exposure duration and repetition, exposure route, body weight). In this case, the conversion factor represents the Daily AUC associated with human continuous exposure to 1 mg/kg/day or 1ppm for oral and inhalation exposure, respectively. For continuous drinking water exposure, the animal value would be divided by 479 to obtain an exposure dose (mg/kg/day), which would be multiplied by 70 kg and divided by 2 L/day to obtain the acceptable drinking water concentration. For continuous inhalation exposure, the animal internal dose metric value is divided by 303 to obtain the human exposure

concentration (ppm). For application of the MOE approach, the points of departure would also be divided by the desired margin based upon the factors discussed above.

This analysis is provided in Table 11 for each of the endpoints discussed. In all cases, a factor of 10 for interindividual human variation and sensitive subpopulations has been assumed. It has also been assumed that humans are equally sensitive to rodents for the PPARα-mediated response (*i.e.*, a factor of 1), although it is plausible that humans are signficantly less responsive than rodents. For the points of departure based upon the cancer data, the slope and severity would be included in the MOE potentially with a factor of 10 for each. This would give a composite desired MOE of 1000. However, the mode of action analysis presented here suggests that a combined factor of 10 would be adequately protective. This choice is based upon the analysis indicating that doses giving little or no substantial increase in LW/BW would be inadequate to induce cancer. Alternatively, the point of departure could be estimated directly from the LW/BW change. In this case, a composite desired MOE of 100 would be appropriate, incorporating factors of 10 for human variability and 10 to insure adequate protection from the potential cancer response and any uncertainties in the shape of the dose-response curve at low dose regions.

The results in Table 11 indicate that higher acceptable exposure levels are estimated using the LW/BW than the cancer endpoint. As indicated in the discussion of the points of departure listed in Table 10, the lower points of departure for cancer following oral dosing reflect the uncertainties of extrapolation below the doses used for measurements. By contrast, the LW/BW data was obtained over a wide range of doses, allowing interpolation. Thus, this report argues that from the view point of mechanism and superior dose response data, LW/BW is an appropriate early indicator which if minimized would prevent the development of liver cancer.

CONCLUSIONS

This analysis identified key limitations of current data. In the area of pharmacokinetics, the production of TCA from TCE following corn oil gavage dosing needs to be better

characterized, as this is critical for setting the oral uptake parameters. There is very limited TCA pharmacokinetic data, which appeared to show dose dependencies in the volume of distribution. Better data would be desirable for estimating the dose metrics in the TCA drinking water studies. The major problem with the carcinogenicity data is that the studies use such a wide variety of exposure regimens, durations, and time between dosing and evaluating cancer. For the PPARα-mediated mechanism, these factors will lead to varying degrees of tumor regression compared to continuous exposure and termination of the study simultaneously with terminating dosing. A notable data gap is the lack of a lifetime study in male B6C3F1 mice exposed to TCA.

This analysis has organized and analyzed the available data on the mode of action for induction of mouse liver tumors by trichloroethylene and perchloroethylene. This analysis has been guided by a simple four step framework for biologically-motivated dose-response analysis: 1) exposure, 2) tissue dosimetry/pharmacokinetics 3) toxicity process/pharmacodynamics, and 4) response. Within these four parts, varying methods were applied depending upon the available information and those factors that appear important determinants of the mode of action. Thus, physiologically-based pharmacokinetic models can be used for estimating internal doses following exposures to TCE, TCA, and PERC. The qualitative information describing the process leading to toxicity indicates that PPARα activation resulting in a pleiotrophic response evident in increased LW/BW is required for the induction of liver carcinogenesis by TCE and TCA. For PERC, it appears an additional process impacts the development of toxicity, which is correlated with, if not directly resulting from, the induction of fatty lipid apparently by the parent compound. Options for empirical analysis were explored using the cancer data and the LW/BW ratio, which provides information over a much broader dose-range that the cancer data.

REFERENCES

Andersen, M. E. and Barton, H. A. (1999). Biological Regulation of Receptor-Hormone Complex Concentrations in Relation to Dose Response Assessments for Endocrine Active Compounds. *Toxicol. Sci.* **48**, 38-50.

Andersen, M. E., Birnbaum, L. S., Barton, H. A. and Eklund, C. (1997a). Regional Hepatic CYP1A1 and CYP1A2 Induction with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin Evaluated with a Multi-Compartment Geometric Model of Hepatic Zonation. *Toxicol. Appl. Pharmacol.* **144**, 145-155.

Andersen, M. E. and Conolly, R. B. (1998). Mechanistic Modeling of Rodent Liver Tumor Promotion at Low Levels of Exposure: An Example Related to Dose-Response Relationships for 2,3,7,8-Tetrachlorodibenzo-p-dioxin. *Hum. Exp. Toxicol.* **17**, 683-690.

Andersen, M. E., Eklund, C. R., Mills, J. J., Barton, H. A. and Birnbaum, L. S. (1997b). A Multi-Compartment Geometric Model of the Liver in Relation to Regional Induction of Cytochrome P450s. *Toxicol. Appl. Pharmacol.* **144**, 135-144.

Andersen, M. E. and Jenkins, L. J., Jr. (1977). Oral Toxicity of 1,1-Dichloroethylene in the Rat: Effects of Sex, Age, and Fasting. *Environ. Health Perspect.* **21**, 157-163.

Andersen, M. E., Mills, J. J., Jirtle, R. L. and Greenlee, W. F. (1995). Negative Selection in Hepatic Tumor Promotion in Relation to Cancer Risk Assessment. *Toxicology* **102**, 223-237.

Aoyama, T., Peters, J., Iritani, N., Nakajima, T., Furihata, K., Hashimoto, T. and Gonzalez, F. (1998). Altered constitutive expression of fatty acid-metabolizing enzymes in mice lacking the peroxisome proliferator-activated receptor alpha (PPARalpha). *J. Biol. Chem.* **273**, 5678-5684.

Ashby, J., Brady, A., Elcombe, C., Elliott, B., Ishmael, J., Odum, J., Tugwood, J., Kettle, S. and Purchase, I. (1994). Mechanistically-based human hazard assessment of peroxisome proliferator-induced hepatocarcinogenesis. *Hum. Exp. Toxicol.* **13**, S1-S117.

ATSDR (1996). *Toxicological Profile for Tetrachloroethylene*. CRC Press LLC, Boca Raton, FL.

ATSDR (1997a). *ATSDR's Toxicological Profiles on CD-ROM.* CRC Press LLC, Boca Raton, FL.

ATSDR (1997b). *Toxicological Profile for Trichloroethylene - Update.*Agency for Toxic Substances and Disease Registry, PB98-101165, Atlanta, GA

Auboeuf, D., Rieusset, J., Fajas, L., Vallier, P., Frering, V., Riou, J., Staels, B., Auwerx, J., Laville, M. and Vidal, H. (1997). Tissue distribution and quantification of the expression of mRNAs of peroxisome proliferator-activated receptors and liver X receptor-alpha in humans: no alteration in adipose tissue of obese and NIDDM patients. *Diabetes* **46**, 1319-1327.

Austin, E. W., Okita, J. R., Okita, R. T., Larson, J. L. and Bull, R. J. (1995). Modification of Lipoperoxidative Effects of Dichloroacetate and Trichloroacetate Is Associated with Peroxisome Proliferation. *Toxicology* **97**, 59-69.

Barrass, N., Price, R., Lake, B. and Orton, T. (1993). Comparison of the acute and chronic mitogenic effects of the peroxisome proliferators methylclofenapate and clofibric acid in rat liver. *Carcinogenesis* **14**, 1451-1456.

Bars, R., Bell, D. and Elcombe, C. (1993). Induction of cytochrome P450 and peroxisomal enzymes by clofibric acid in vivo and in vitro. *Biochem. Pharmacol.* **45**, 2045-2053.

Barton, H. A., Bull, R., Schultz, I. and Andersen, M. E. (1999). Dichloroacetate (DCA) dosimetry: Interpreting DCA-induced liver cancer dose response and the potential for DCA to contribute to trichloroethylene-induced liver cancer. *Toxicol. Lett.* **106**, 9-21.

Barton, H. A. and Clewell, H. J., III (1998). *Noncancer Effects Due to Trichloroethylene: Pharmacokinetics and Risk Assessment*.USAF Armstrong Laboratory, AL-OE-BR-TR-1998-0029, Brooks AFB, TX

Barton, H. A. and Das, S. (1996). Alternatives for a Risk Assessment on Chronic Noncancer Effects from Oral Exposure to Trichloroethylene. *Regul. Toxicol. Pharmacol.* **24**, 269-285.

Beier, K., Volkl, A., Metzger, C., Mayer, D., Bannasch, P. and Fahimi, H. (1997). Hepatic zonation of the induction of cytochrome P450 IVA, peroxisomal lipid beta-oxidation enzymes and peroxisome proliferation in rats treated with dehydroepiandrosterone (DHEA). Evidence of distinct zonal and sex-specific differences. *Carcinogenesis* **18**, 1491-1498.

Bell, D., Bars, R. and Elcombe, C. (1992). Differential tissue-specific expression and induction of cytochrome P450IVA1 and acyl-CoA oxidase. *Eur. J. Biochem.* **206**, 979-986.

Bell, D. and Elcombe, C. (1991). Induction of acyl-CoA oxidase and cytochrome P450IVA1 RNA in rat primary hepatocyte culture by peroxisome proliferators. *Biochem. J.* **280**, 249-253.

Bell, D. R., Bars, R. G., Gibson, G. G. and Elcombe, C. R. (1991). Localization and Differential Induction of Cytochrome *P*450IVa and Acyl-CoA Oxidase in Rat Liver. *Biochem. J.* **275**, 247-252.

Bell, Z., Olson, K., and Benya, T., (1978). *Final Report of Audit Findings of the Manufacturing Chemists Association (MCA): Administered Trichloroethylene (TCE) Chronic Inhalation Study*.Industrial Bio-Test Laboratories, Inc., Decatur, IL

Belury, M., Moya-Camarena, S., Sun, H., Snyder, E., Davis, J., Cunningham, M. and Vanden Heuvel, J. (1998). Comparison of dose-response relationships for induction of lipid metabolizing and growth regulatory genes by peroxisome proliferators in rat liver. *Toxicol. Appl. Pharmacol.* **151**, 254-261.

Berman, E., Schlicht, M., Moser, V. and MacPhail, R. (1995). A Multidisciplinary Approach to Toxicological Screening: I. Systemic Toxicity. *J. Toxicol. Environ. Health* **45**, 127-143.

Blaauboer, B., van Holsteijn, C., Bleumink, R., Mennes, W., van Pelt, F., Yap, S., van Pelt, J., van Iersel, A., Timmerman, A. and Schmid, B. (1990). The effect of beclobric acid and clofibric acid on peroxisomal beta-oxidation and peroxisome proliferation in primary cultures of rat, monkey and human hepatocytes. *Biochem. Pharmacol.* **40**, 521-528.

Bocos, C., Göttlicher, M., Gearing, K., Banner, C., Enmark, E., Teboul, M., Crickmore, A. and Gustafsson, J.-Å. (1995). Fatty Acid Activation of Peroxisome Proliferator-Activated Receptor (PPAR). *J. Steroid Biochem. Mol. Biol.* **53**, 467-473.

Bogen, K. T. (1988). Pharmacokinetics for Regulatory Risk Analysis: The Case of Trichloroethylene. *Regul. Toxicol. Pharmacol.* **8**, 447-466.

Bogen, K. T. and Gold, L. S. (1997). Trichloroethylene Cancer Risk: Simplified Calculation of PBPK-Based MCLs for Cytotoxic End Points. *Regul. Toxicol. Pharmacol.* **25**, 26-42.

Bojes, H., Germolec, D., Simeonova, P., Bruccoleri, A., Schoonhoven, R., Luster, M. and Thurman, R. (1997). Antibodies to tumor necrosis factor alpha prevent increases in cell replication in liver due to the potent peroxisome proliferator, WY-14,643. *Carcinogenesis* **18**, 669-674.

Bojes, H. and Thurman, R. (1996). Peroxisome proliferators activate Kupffer cells in vivo. *Cancer Res.* **56**, 1-4.

Braissant, O., Foufelle, F., Scotto, C., Dauca, M. and Wahli, W. (1996). Differential expression of peroxisome proliferator-activated receptors (PPARs): tissue distribution of PPAR-alpha, -beta, and -gamma in the adult rat. *Endocrinology* **137**, 354-366.

Bruckner, J. V., Davis, B. D. and Blancato, J. N. (1989). Metabolism, Toxicity and Carcinogenicity of Trichloroethylene. *Crit. Rev. Toxicol.* **20**, 31-50.

Bruning, T., Golka, K., Makropoulos, V. and Bolt, H. M. (1996). Preexistence of Chronic Tubular Damage in Cases of Renal Cell Cancer After Long and High Exposure to Trichloroethylene. *Arch. Toxicol.* **70**, 259-260.

Bruning, T., Vamvakas, S., Makropoulos, V. and Birner, G. (1998). Acute Intoxication with Trichloroethene: Clinical Symptoms, Toxicokinetics, Metabolism and Development of Biochemical Parameters for Renal Damage. *Toxicol. Sci.* **41**, 157-165.

Bruning, T., Weirich, G., Hornauer, M. A., Hofler, H. and Brauch, H. (1997). Renal Cell Carcinomas in Trichloroethene (TRI) Exposed Persons are Associated with Somatic Mutations in the von Hippel-Lindau (VHL) Tumour Suppressor Gene. *Arch. Toxicol.* **71**, 332-335.

Brzozowski, A., Pike, A., Dauter, Z., Hubbard, R., Bonn, T., Engstrom, O., Ohman, L., Greene, G., Gustafsson, J. and Carlquist, M. (1997). Molecular basis of agonism and antagonism in the oestrogen receptor. *Nature* **389**, 753-758.

Buben, J. A. and O'Flaherty, E. J. (1985). Delineation of the Role of Metabolism in the Hepatotoxicity of Trichloroethylene and Perchloroethylene: A Dose-Effect Study. *Toxicol. Appl. Pharmacol.* **78**, 105-122.

Budroe, J., Umemura, T., Angeloff, K. and Williams, G. (1992). Dose-response relationships of hepatic acyl-CoA oxidase and catalase activity and liver mitogenesis induced by the peroxisome proliferator ciprofibrate in C57BL/6N and BALB/c mice. *Toxicol. Appl. Pharmacol.* **113**, 192-198.

Bull, R. J., Sanchez, I. M., Nelson, M. A., Larson, J. L. and Lansing, A. J. (1990). Liver Tumor Induction in B6C3F1 Mice by Dichloroacetate and Trichloroacetate. *Toxicology* **63**, 341-359.

Butterworth, B., Templin, M., Borghoff, S., Conolly, R., Kedderis, G. and Wolf, D. (1995). The role of regenerative cell proliferation in chloroform-induced cancer. *Toxicol. Lett.* **82-83**, 23-26.

Cattley, R., DeLuca, J., Elcombe, C., Fenner-Crisp, P., Lake, B., Marsman, D., Pastoor, T., Popp, J., DE, R., Schwetz, B., Tugwood, J. and Wahli, W. (1998). Do peroxisome proliferating compounds pose a hepatocarcinogenic hazard to humans? *Regul. Toxicol. Pharmacol.* **27**, 47-60.

Cattley, R., Marsman, D. and Popp, J. (1991). Age-related susceptibility to the carcinogenic effect of the peroxisome proliferator WY-14,643 in rat liver. *Carcinogenesis* **12**, 469-473.

Channel, S., Latendresse, J., Kidney, J., Grabau, J., Lane, J., Steel-Goodwin, L. and Gothaus, M. (1998). A subchronic exposure to trichloroethylene causes lipid peroxidation and hepatocellular proliferation in male B6C3F1 mouse liver. *Toxicol. Sci.* **43**, 145-154.

Chen, Z., White, C., He, C., Liu, Y. and Eaton, D. (1995). Zonal differences in DNA synthesis activity and cytochrome P450 gene expression in livers of male F344 rats treated with five nongenotoxic carcinogens. *J. Environ. Pathol. Toxicol. Oncol.* **14**, 83-99.

Christensen, J., Gonzales, A., Cattley, R. and Goldsworthy, T. (1998). Regulation of apoptosis in mouse hepatocytes and alteration of apoptosis by nongenotoxic carcinogens. *Cell Growth Differ.* **9**, 815-825.

Chu, S., Huang, Q., Alvares, K., Yeldandi, A., Rao, M. and Reddy, J. (1995). Transformation of mammalian cells by overexpressing H2O2-generating peroxisomal fatty acyl-CoA oxidase. *Proc. Natl. Acad. Sci. USA* **92**, 7080-7084.

Citron, M. (1995). Perplexing peroxisome proliferators. *Environ. Health Perspect.* **103**, 232-235.

Clewell, H., Gentry, P., Gearhart, J., Allen, B. and Andersen, M. (1995). Considering Pharmacokinetic and Mechanistic Information in Cancer Risk Assessments for Environmental Contaminants: Examples with Vinyl Chloride and Trichloroethylene. *Chemosphere* **31**, 2561-2578.

Clewell, H. J., III (1995). The Use of Physiologically Based Pharmacokinetic Modeling in Risk Assessment: A Case Study with Methylene Chloride. In *Low-Dose Extrapolation of Cancer Risks: Issues and Perspectives.*, Olin, S., Farland, W., Park, C., Rhomberg, L., Scheuplein, R., Starr, T. and Wilson, J., Eds., ILSI Press, Washington, DC.

Clewell, H. J., III, Gentry, P. R., Covington, T. R. and Gearhart, J. M. (1999). Development of a Physiologically-Based Pharmacokinetic Model of Trichloroethylene and Its Metabolites for Use in Risk Assessment. *Environ. Health Perspect.* , submitted.

Clewell, H. J., III, Gentry, P. R., Gearhart, J. M. and Allen, B. C. (1994). *Evaluation of Pharmacokinetic Models for Use in Cancer Risk Assessment: Example with Trichloroethylene*.OSHA Directorate of Health Standards Programs and EPA Office of Health and Environmental Assessment, ,

Cogliano, J. (1999). Trichloroethylene health risk assessment: Using mechanistic information to improve dose-response assessment. *Toxicologist* **48**, Abstr # 387.

Daniel, F. B., DeAngelo, A. B., Stober, J. A., Olson, G. R. and Page, N. P. (1992). Hepatocarcinogenicity of Chloral Hydrate, 2-Chloroacetaldehyde and Dichloroacetic Acid in the Male B6C3F1 Mouse. *Fundam. Appl. Toxicol.* **19**, 159-168.

DeAngelo, A., Daniel, F., Most, B. and Olson, G. (1997). Failure of monochloroacetic acid and trichloroacetic acid administered in the drinking water to produce liver cancer in male F344/N rats. *J. Toxicol. Environ. Health* **52**, 425-445.

DeAngelo, A. and George, M. (1995). Evaluation of the carcingenicity of chloral hydrate in the male B6C3F1 mouse and F344 rat. *Proc. Am. Assoc. for Cancer Res.* **36**, 132.

DeAngelo, A. B. and Daniel, F. B. (1990). The comparative carcinogenicity of dichloroacetic (DCA) and trichloroacetic (TCA) acid in the male B6C3F1 mouse. *Toxicologist* **10**, Abstr# 592.

DeAngelo, A. B., Daniel, F. B., McMillan, L., Wernsing, P. and Savage, R. E., Jr. (1989). Species and Strain Sensitivity to the Induction of Peroxisome Proliferation by Chloroacetic Acids. *Toxicol. Appl. Pharmacol.* **101**, 285-298.

Dees, C. and Travis, C. (1993). The Mitogenic Potential of Trichloroethylene in B6C3F1 Mice. *Toxicol. Lett.* **69**, 129-137.

Dees, C. and Travis, C. (1994). Trichloroacetate Stimulation of Liver DNA Synthesis in Male and Female Mice. *Toxicol. Lett.* **70**, 343-355.

Dekant, W., Koob, M. and Henschler, D. (1990). Metabolism of Trichloroethene--In Vivo and in Vitro Evidence for Activation by Glutathione Conjugation. *Chem.-Biol. Interact.* **73**, 89-101.

Dekant, W., Martens, G., Vamvakas, S., Metzler, M. and Henschler, D. (1987). Bioactivation of Tetrachloroethylene. Role of Glutathione S-Transferase-Catalyzed Conjugation versus Cytochrome P-450-Dependent Phospholipid Alkylation. *Drug Metab. Dispos.* **15**, 702-709.

Dekant, W., Metzler, M. and Henschler, D. (1986). Identification of S-1,2-Dichlorovinyl-N-Acetyl-Cysteine as a Urinary Metabolite of Trichloroethylene: A Possible Explanation for its Nephrocarcinogenicity in Male Rats. *Biochem. Pharmacol.* **35**, 2455-2458.

Dekant, W., Vamvakas, S. and Anders, M. (1989). Bioactivation of nephrotoxic haloalkenes by glutathione conjugation: formation of toxic and mutagenic intermediates by cysteine conjugate beta-lyase. *Drug Metab. Rev.* **20**, 43-83.

Dirven, H., van den Broek, P., Peeters, M., Peters, J., Mennes, W., Blaauboer, B., Noordhoek, J. and Jongeneelen, F. (1993). Effects of the peroxisome proliferator mono(2-ethylhexyl)phthalate in primary hepatocyte cultures derived from rat, guinea pig, rabbit and monkey. Relationship between interspecies differences in biotransformation and peroxisome proliferating potencies. *Biochem. Pharmacol.* **45**, 2425-2434.

Elcombe, C. R. (1985). Species Differences in Carcinogenicity and Peroxisome Proliferation Due to Trichloroethylene: a Biochemical Human Hazard Assessment. *Arch. Toxicol. Suppl.* **8**, 6-17.

Elcombe, C. R., Rose, M. S. and Pratt, I. S. (1985). Biochemical, Histological, and Ultrastructural Changes in Rat and Mouse Liver Following the Administration of Trichloroethylene: Possible Relevance to Species Differences in Hepatocarcinogenicity. *Toxicol. Appl. Pharmacol.* **79**, 365-376.

EPA (1985). *Health Assessment Document for Trichloroethylene, Final Report.*US Environmental Protection Agency, EPA/600/8-82/006F, Washington,DC

EPA (1986). Guidelines for Carcinogen Risk Assessment. *Fed. Regist.* **51**, 33992.

EPA (1996). *Proposed Guidelines for Carcinogen Risk Assessment.*Office of Research and Development, EPA 600-P-92-003C, Washington, DC

Fahimi, H., Beier, K., Lindauer, M., Schad, A., Zhan, J., Pill, J., Rebel, W., Volkl, A. and Baumgart, E. (1996). Zonal heterogeneity of peroxisome proliferation in rat liver. *Ann. N. Y. Acad. Sci.* **804**, 341-361.

Fan, C., Pan, J., Usuda, N., Yeldandi, A., Rao, M. and Reddy, J. (1998). Steatohepatitis, spontaneous peroxisome proliferation and liver tumors in mice lacking peroxisomal fatty acyl-CoA oxidase. Implications for peroxisome proliferator-activated receptor alpha natural ligand metabolism. *J. Biol. Chem.* **273**, 15639-15645.

Fenner-Crisp, P. (1996). Regulatory implications: U.S. Environmental Protection Agency. *Ann. N. Y. Acad. Sci.* **804**, 636-640.

Ferguson, L., Morcombe, P. and Triggs, C. (1993). The size of cytokinesis-blocked micronuclei in human peripheral blood lymphocytes as a measure of aneuploidy induction by Set A compounds in the EEC trial. *Mutat. Res.* **287**, 101-112.

Ferreira-Gonzalez, A., DeAngelo, A. B., Nasim, S. and garrett, C. T. (1995). *Ras* Oncogene Activation During Hepatocarcinogensis in B6C3F1 Male Mice by Dichloroacetic and Trichloroacetic Acids. *Carcinogenesis* **16**, 495-500.

Fisher, J., Gargas, M., Allen, B. and Andersen, M. (1991). Physiologically Based Pharmacokinetic Modeling with Trichloroethylene and Its Metabolite, Trichloroacetic Acid, in the Rat and Mouse. *Toxicol. Appl. Pharmacol.* **109**, 183-195.

Foxworthy, P., White, S., Hoover, D. and Eacho, P. (1990). Effect of ciprofibrate, bezafibrate, and LY171883 on peroxisomal beta-oxidation in cultured rat, dog, and rhesus monkey hepatocytes. *Toxicol. Appl. Pharmacol.* **104**, 386-394.

Fukuda, K., Takemoto, K. and Tsuruta, H. (1983). Inhalation Carcinogenicity of Trichloroethylene in Mice and Rats. *Ind. Health* **21**, 243-254.

Gearhart, J., Mahle, D., Greene, R., Seckel, C., Flemming, C., Fisher, J. and Clewell, H., 3d (1993). Variability of physiologically based pharmacokinetic (PBPK) model parameters and their effects on PBPK model predictions in a risk assessment for perchloroethylene (PCE). *Toxicol. Lett.* **68**, 131-144.

Geiss, K., Grabau, J., Lane, J. and Latendresse, J. (1997). *Quantitative Assessment of Peroxisome Proliferation in B6C3F1 Mouse Liver After Subchronic Exposure to Trichloroethylene by Gavage*.Occupational and Environmental Health Directorate, Toxicology Division, Wright Patterson AFB, AL/OE-TR-1997-0087,

Gibson, G. (1992). Co-induction of cytochrome P4504A1 and peroxisome proliferation: a causal or casual relationship? *Xenobiotica* **22**, 1101-1109.

Gibson, G. (1996). Peroxisome proliferators and cytochrome P4504A induction. *Ann. N. Y. Acad. Sci.* **804**, 328-340.

Gill, J., Brickell, P., Dive, C. and Roberts, R. (1998a). The rodent non-genotoxic hepatocarcinogen nafenopin suppresses apoptosis preferentially in non-cycling hepatocytes but also elevates CDK4, a cell cycle progression factor. *Carcinogenesis* **19**, 1743-1747.

Gill, J., James, N., Roberts, R. and Dive, C. (1998b). The non-genotoxic hepatocarcinogen nafenopin suppresses rodent hepatocyte apoptosis induced by TGFbeta1, DNA damage and Fas. *Carcinogenesis* **19**, 299-304.

Goeptar, A. R., Commandeur, J. N. M., van Ommen, B., van Bladeren, P. J. and Vermeulen, N. P. E. (1995). Metabolism and Kinetics of Trichloroethylene in Relation to Toxicity and Carcinogenicity. Relevance of the Mercapturic Acid Pathway. *Chem. Res. Toxicol.* **8**, 3-21.

Goldsworthy, T. L. and Popp, J. A. (1987). Chlorinated Hydrocarbon-Induced Peroxisomal Enzyme Activity in Relation to Species and Organ Carcinogenicity. *Toxicol. Appl. Pharmacol.* **88**, 225-233.

Gonzalez, F. (1997). Recent update on the PPAR alpha-null mouse. *Biochimie* **79**, 139-144.

Gonzalez, F. J., Peters, J. M. and Cattley, R. C. (1998). Mechanism of Action of the Nongenotoxic Peroxisome Proliferators: Role of the Peroxisome Proliferator-Activated Receptor α. *J. Natl. Cancer Inst.* **90**, 1702-1709.

Gottlicher, M., Widmark, E., Li, Q. and Gustafsson, J. (1992). Fatty acids activate a chimera of the clofibric acid-activated receptor and the glucocorticoid receptor. *Proc. Natl. Acad. Sci. USA* **89**, 4653-4657.

Graham, M., Wilson, S., Winham, M., Spencer, A., Rees, J., Old, S. and Bonner, F. (1994). Lack of peroxisome proliferation in marmoset liver following treatment with ciprofibrate for 3 years. *Fundam. Appl. Toxicol.* **22**, 58-64.

Grasl-Kraupp, B., Ruttkay-Nedecky, B., Mullauer, L., Taper, H., Huber, W., Bursch, W. and Schulte-Hermann, R. (1997). Inherent increase of apoptosis in liver tumors: implications for carcinogenesis and tumor regression. *Hepatology* **25**, 906-912.

Hasmall, S., James, N., Soames, A. and Roberts, R. (1998). The peroxisome proliferator nafenopin does not suppress hepatocyte apoptosis in guinea-pig liver in vivo nor in human hepatocytes in vitro. *Arch. Toxicol.* **72**, 777-783.

Henschler, D. (1984). Exposure limits: history, philosophy, future developments. *Ann. Occup. Hyg.* **28**, 79-82.

Henschler, D., Romen, W., Elsasser, H. M., Reichert, D., Eder, E. and Radwan, Z. (1980). Carcinogenicity Study of Trichloroethylene by Longterm Inhalation in Three Animal Species. *Arch. Toxicol.* **43**, 237-248.

Herren-Freund, S. L., Pereira, M. A., Khoury, M. D. and Olson, G. (1987). The Carcinogenicity of Trichloroethylene and its Metabolites, Trichloroacetic Acid and Dichloroacetic Acid, in Mouse Liver. *Toxicol. Appl. Pharmacol.* **90**, 183-189.

Holloway, B. R., Bentley, M. and Thorp, J. M. (1982a). Species differences in the effects of ICI 55,897 on plasma lipids and hepatic peroxisomes. *Ann. N. Y. Acad. Sci.* **386**, 439-442.

Holloway, B. R., Thorp, J. M., Smith, G. D. and Peters, T. J. (1982b). Analytical subcellular fractionation and enzymic analysis of liver homogenates from control and clofibrate-treated rats, mice, and monkeys with reference to the fatty acid-oxidizing enzymes. *Ann. N. Y. Acad. Sci.* **386**, 439-442.

Issemann, I. and Green, S. (1990). Activation of a member of the steroid hormone receptor superfamily by peroxisome proliferators. *Nature* **347**, 645-650.

James, N. and Roberts, R. (1994). The peroxisome proliferator class of non-genotoxic hepatocarcinogens synergize with epidermal growth factor to promote clonal expansion of initiated rat hepatocytes. *Carcinogenesis* **15**, 2687-2694.

James, N. and Roberts, R. (1995). Species differences in the clonal expansion of hepatocytes in response to the coaction of epidermal growth factor and nafenopin, a rodent hepatocarcinogenic peroxisome proliferator. *Fundam. Appl. Toxicol.* **26**, 143-149.

Johnson, E., Palmer, C., Griffin, K. and Hsu, M. (1996a). Role of the peroxisome proliferator-activated receptor in cytochrome P450 4A gene regulation. *FASEB J.* **10**, 1241-1248.

Johnson, E., Palmer, C. and Hsu, M. (1996b). The peroxisome proliferator-activated receptor: Transcriptional activation of the CYP4A6 gene. *Ann. N. Y. Acad. Sci.* **804**, 373-386.

Kaikaus, R., Chan, W., Lysenko, N., Ray, R., Ortiz de Montellano, P. and Bass, N. (1993). Induction of peroxisomal fatty acid beta-oxidation and liver fatty acid-binding protein by peroxisome proliferators. Mediation via the cytochrome P-450IVA1 omega-hydroxylase pathway. *J. Biol. Chem.* **268**, 9593-9603.

Katzenellenbogen, J. A., O'Malley, B. W. and Katzenellenbogen, B. S. (1996). Tripartite Steroid Hormone Receptor Pharmacology: Interaction with Multiple Effector Sites as a Basis for the Cell- and Promoter-Specific Action of These Hormones. *Mol. Endocrinol.* **10**, 119-131.

Keller, H., Devchand, P., Perroud, M. and Wahli, W. (1997). PPAR alpha structure-function relationships derived from species-specific differences in responsiveness to hypolipidemic agents. *Biol. Chem.* **378**, 651-655.

Ketcha, M. M., Stevens, D. K., Warren, D. A., Bishop, C. T. and Brashear, W. T. (1996). Conversion of Trichloroacetic Acid to Dichloroacetic Acid in Biological Samples. *J. Anal. Toxicol.* **20**, 236-241.

Kimura, S., Hardwick, J., Kozak, C. and Gonzalez, F. (1989). The rat clofibrate-inducible CYP4A subfamily. II. cDNA sequence of IVA3, mapping of the Cyp4a locus to mouse chromosome 4, and coordinate and tissue-specific regulation of the CYP4A genes. *DNA* **8**, 517-525.

Kjellstrand, P., Holmquist, B., Alm, P., Kanje, M., Romare, S., Jonsson, I., Mansson, L. and Bjerkemo, M. (1983). Trichloroethylene: Further Studies of the Effects on Body and Organ Weights and Plasma Butyrylcholinesterase Activity in Mice. *Acta Pharmacol. Toxicol.* **53**, 375-384.

Kjellstrand, P., Holmquist, B., Kanje, M., Alm, P., Romare, S., Jonsson, I., Mansson, L. and Bjerkemo, M. (1984). Perchloroethylene: Effects on Body and Organ Weights and Plasma Butyrylcholinesterase Activity in Mice. *Acta Pharmacol. Toxicol.* **54**, 414-424.

Kjellstrand, P., Kanje, M., Mansson, L., Bjerkemo, M., Mortensen, I., Lanke, J. and Holmquist, B. (1981). Trichloroethylene: Effects on Body and Organ Weights in Mice, Rats and Gerbils. *Toxicology* **21**, 105-115.

Kraupp-Grasl, B., Huber, W., Putz, B., Gerbracht, U. and Schulte-Hermann, R. (1990). Tumor promotion by the peroxisome proliferator nafenopin involving a specific subtype of altered foci in rat liver. *Cancer Res.* **50**, 3701-3708.

Kraupp-Grasl, B., Huber, W., Taper, H. and Schulte-Hermann, R. (1991). Increased susceptibility of aged rats to hepatocarcinogenesis by the peroxisome proliferator nafenopin and the possible involvement of altered liver foci occurring spontaneously. *Cancer Res.* **51**, 666-671.

Krey, G., Braissant, O., L'Horset, F., Kalkhoven, E., Perroud, M., Parker, M. G. and Wahli, W. (1997). Fatty acids, eicosanoids, and hypolipidemic agents identified as ligands of peroxisome proliferator-activated receptors by coactivator-dependent receptor ligand assay. *Mol. Endocrinol.* **11**, 779-791.

Kurata, Y., Kidachi, F., Yokoyama, M., Toyota, N., Tsuchitani, M. and Katoh, M. (1998). Subchronic toxicity of Di(2-ethylhexyl)phthalate in common marmosets: lack of hepatic peroxisome proliferation, testicular atrophy, or pancreatic acinar cell hyperplasia. *Toxicol. Sci.* **42**, 49-56.

Lake, B., Charzat, C., Tredger, J., Renwick, A., Beamand, J. and Price, R. (1996). Induction of cytochrome P450 isoenzymes in cultured precision-cut rat and human liver slices. *Xenobiotica* **26**, 297-306.

Larson, J. L. and Bull, R. J. (1992a). Metabolism and Lipoperoxidative Activity of Trichloroacetate and Dichloroacetate in Rats and Mice. *Toxicol. Appl. Pharmacol.* **115**, 268-277.

Larson, J. L. and Bull, R. J. (1992b). Species Differences in the Metabolism of Trichloroethylene to the Carcinogenic Metabolites Trichloroacetate and Dichloroacetate. *Toxicol. Appl. Pharmacol.* **115**, 278-285.

Latendresse, J. R. and Pereira, M. A. (1997). Dissimilar Characteristics of *N*-Methyl-*N*-Nitrosourea-Initiated Foci and Tumors Promoted by Dichloroacetic Acid or Trichloroacetic Acid in the Liver of Female B6C3F1 Mice. *Toxicol. Pathol.* **25**, 433-440.

Lemberger, T., Braissant, O., Juge-Aubry, C., Keller, H., Saladin, R., Staels, B., Auwerx, J., Burger, A., Meier, C. and Wahli, W. (1996). PPAR tissue distribution and interactions with other hormone-signaling pathways. *Ann. N. Y. Acad. Sci.* **804**, 231-251.

Lemberger, T., Staels, B., Saladin, R., Desvergne, B., Auwerx, J. and Wahli, W. (1994). Regulation of the peroxisome proliferator-activated receptor alpha gene by glucocorticoids. *J. Biol. Chem.* **269**, 24527-24530.

Lin, Q., Ruuska, S., Shaw, N., Dong, D. and Noy, N. (1999). Ligand selectivity of the peroxisome proliferator-activated receptor alpha. *Biochemistry* **38**, 186-190.

Lindauer, M., Beier, K., Volkl, A. and Fahimi, H. (1994). Zonal heterogeneity of peroxisomal enzymes in rat liver: differential induction by three divergent hypolipidemic drugs. *Hepatology* **20**, 475-486.

Maltoni, C., Lefemine, G. and Cotti, G., Eds. (1986). *Experimental Research on Trichloroethylene Carcinogenesis*. Archives of Research on Industrial Carcinogenesis. Princeton Scientific Publishing, Princeton, NJ.

Maltoni, C., Lefemine, G., Cotti, G. and Perino, G. (1988). Long-Term Carcinogenicity Bioassays on Trichloroethylene Administered by Inhalation to Sprague-Dawley Rats and Swiss and B6C3F1 Mice. *Ann. N. Y. Acad. Sci.* **534**, 317-342.

Maronpot, R., Fox, T., Malarkey, D. and Goldsworthy, T. (1995). Mutations in the ras proto-oncogene: clues to etiology and molecular pathogenesis of mouse liver tumors. *Toxicology* **101**, 125-156.

Marsman, D., Goldsworthy, T. and Popp, J. (1992). Contrasting hepatocytic peroxisome proliferation, lipofuscin accumulation and cell turnover for the hepatocarcinogens Wy-14,643 and clofibric acid. *Carcinogenesis* **13**, 1011-1017.

Marsman, D. and Popp, J. (1994). Biological potential of basophilic hepatocellular foci and hepatic adenoma induced by the peroxisome proliferator, Wy-14,643. *Carcinogenesis* **15**, 111-117.

Marsman, D. S., Cattley, R. C., Conway, J. G. and Popp, J. A. (1988). Relationship of Hepatic Peroxisome Proliferation and Replicative DNA Synthesis to the Hepatocarcinogenicity of the Peroxisome Proliferators Di(2-ethylhexyl)phthalate and (4-Chloro-6-(2,3-xylidino)-2-pyrimidinylthio)acetic Acid (Wy-14,643) in Rats. *Cancer Res.* **48**, 6739-6744.

Mather, G. G., Exon, J. H. and Koller, L. D. (1990). Subchronic 90 Day Toxicity of Dichloroacetic and Trichloroacetic Acid in Rats. *Toxicology* **64**, 71-80.

Melnick, R., Kohn, M. and Portier, C. (1996). Implications for risk assessment of suggested nongenotoxic mechanisms of chemical carcinogenesis. *Environ. Health Perspect.* **104**, 123-134.

Mennes, W., Wortelboer, H., Hassing, G., van Sandwijk, K., Timmerman, A., Schmid, B., Jahn, U. and Blaauboer, B. (1994). Effects of clofibric and beclobric acid in rat and monkey hepatocyte primary culture: influence on peroxisomal and mitochondrial beta-oxidation and the activity of catalase, glutathione S-transferase and glutathione peroxidase. *Arch. Toxicol.* **68**, 506-511.

Merdink, J. L., Gonzalez-Leon, A., Bull, R. J. and Shultz, I. R. (1998). The Extent of Dichloroacetate Formation from Trichloroethlyene, Chloral Hydrate, Trichloroacetate and Trichloroethanol in B6C3F1 Mice. *Toxicol. Sci.* **45**, 33-41.

Merrick, B. A., Robinson, M. and Condie, L. W. (1989). Differing Hepatotoxicity and Lethality After Subacute Trichloroethylene Exposure in Aqueous or Corn Oil Gavage Vehicles in B6C3F1 Mice. *J. Appl. Toxicol.* **9**, 15-21.

Miller, R., Glover, S., Stewart, W., Corton, J., Popp, J. and Cattley, R. (1996). Effect on the expression of c-met, c-myc and PPAR-alpha in liver and liver tumors from rats chronically exposed to the hepatocarcinogenic peroxisome proliferator WY-14,643. *Carcinogenesis* **17**, 1337-1341.

Miller, R. E. and Guengerich, F. P. (1982). Oxidation of Trichloroethylene by Liver Microsomal Cytochrome P-450: Evidence for Chlorine Migration in a Transition State Not Involving Trichloroethylene Oxide. *Biochemistry* **21**, 1090-1097.

Miller, R. E. and Guengerich, F. P. (1983). Metabolism of Trichloroethylene in Isolated Hepatocytes, Microsomes, and Reconstituted Enzyme Systems Containing Cytochrome P-450. *Cancer Res.* **43**, 1145-1152.

Milton, M., Elcombe, C. and Gibson, G. (1990). On the mechanism of induction of microsomal cytochrome P450IVA1 and peroxisome proliferation in rat liver by clofibrate. *Biochem. Pharmacol.* **40**, 2727-2732.

Mukherjee, R., Jow, L., Noonan, D. and McDonnell, D. (1994). Human and rat peroxisome proliferator activated receptors (PPARs) demonstrate similar tissue distribution but different responsiveness to PPAR activators. *J. Steroid Biochem. Mol. Biol.* **51**, 157-166.

NCI (1976). *Carcinogenesis Bioassay of Trichloroethylene*.National Cancer Institute, PB-254 122, Washington, DC

NCI (1977). *Bioassay of Tetrachloroethylene for Possible Carcinogenicity*.National Cancer Institute, PB-272940/AS, Washington, DC

NTP (1983). *Technical Report on the Carcinogenesis Bioassay of Trichloroethylene in F344/N Rats and B6C3F1/N Mice*.National Institute of Environmental Health Sciences, NIH Pub, No, 82-1799, Research Triangle Park, NC

NTP (1986). *Trichloroethylene: Reproduction and Fertility Assessment in F344 Rats When Administered in Feed, Final Report*.National Institutes of Environmental Health Sciences, PB86-190782, Research Triangle Park, NC

NTP (1988). *Toxicology and Carcinogenesis Studies of Trichloroethylene (Cas No, 79-01-6) in Four Strains of Rats (ACI, August, Marshall, Osborne-Mendel) (Gavage studies)*.National Institute of Environmental Health Sciences, PB88-218896, Research Triangle Park, NC

NTP (1990). *Carcinogenesis studies of trichloroethylene (without epichlorohydrinin) (CAS No. 79-01-6) in Fischer-344/N rats and B6C3F1 mice (gavage studies).* Department of Health and Human Services, Public Health Service, National Institute of Health, NIH Publication No. 90-1799, Research Triangle Park, NC

Odum, J., Green, T., Foster, J. R. and Hext, P. M. (1988). The Role of Trichloroacetic Acid and Peroxisome Proliferation in the Differences in Carcinogencity of Perchloroethylene in the Mouse and Rat. *Toxicol. Appl. Pharmacol.* **92**, 103-112.

O'Malley, B. W., Schrader, W. T., Mani, S., Smith, C., Weigel, N. L., Conneely, O. M. and Clark, J. H. (1995). An Alternative Ligand-Independent Pathway for Activation of Steroid Receptors. *Recent Prog. Horm. Res.* **50**, 333-347.

Palmer, C., Hsu, M., Griffin, K., Raucy, J. and Johnson, E. (1998). Peroxisome proliferator activated receptor-alpha expression in human liver. *Mol. Pharmacol.* **53**, 14-22.

Parrish, J., Austin, E., Stevens, D., Kinder, D. and Bull, R. (1996). Haloacetate-induced oxidative damage to DNA in the liver of male B6C3F1 mice. *Toxicology* **110**, 103-111.

Parzefall, W., Erber, E., Sedivy, R. and Schulte-Hermann, R. (1991). Testing for induction of DNA synthesis in human hepatocyte primary cultures by rat liver tumor promoters. *Cancer Res.* **51**, 1143-1147.

Pereira, M., Li, K. and Kramer, P. (1997). Promotion by mixtures of dichloroacetic acid and trichloroacetic acid of N-methyl-N-nitrosourea-initiated cancer in the liver of female B6C3F1 mice. *Cancer Lett.* **115**, 15-23.

Pereira, M. A. (1996). Carcinogenic Activity of Dichloroacetic Acid and Trichloroacetic Acid in the Liver of Female B6C3F1 Mice. *Fundam. Appl. Toxicol.* **31**, 192-199.

Perrone, C., Shao, L. and Williams, G. (1998). Effect of rodent hepatocarcinogenic peroxisome proliferators on fatty acyl-CoA oxidase, DNA synthesis, and apoptosis in cultured human and rat hepatocytes. *Toxicol. Appl. Pharmacol.* **150**, 277-286.

Peters, J., Aoyama, T., Cattley, R., Nobumitsu, U., Hashimoto, T. and Gonzalez, F. (1998). Role of peroxisome proliferator-activated receptor alpha in altered cell cycle regulation in mouse liver. *Carcinogenesis* **19**, 1989-1994.

Peters, J., Hennuyer, N., Staels, B., Fruchart, J., Fievet, C., Gonzalez, F. and Auwerx, J. (1997a). Alterations in lipoprotein metabolism in peroxisome proliferator-activated receptor alpha-deficient mice. *J. Biol. Chem.* **272**, 27307-27312.

Peters, J. M., Cattley, R. C. and Gonzalez, F. J. (1997b). Role of PPARα in the mechanism of action of the nongenotoxic carcinogen and peroxisome proliferator WY-14,643. *Carcinogenesis* **18**, 1910-2033.

Plant, N., Horley, N., Savory, R., Elcombe, C., Gray, T. and Bell, D. (1998). The peroxisome proliferators are hepatocyte mitogens in chemically-defined media: glucocorticoid-induced PPAR alpha is linked to peroxisome proliferator mitogenesis. *Carcinogenesis* **19**, 925-931.

Powell, P., Wolf, I. and Lasker, J. (1996). Identification of CYP4A11 as the major lauric acid omega-hydroxylase in human liver microsomes. *Arch. Biochem. Biophys.* **335**, 219-226.

Prout, M. S., Provan, W. M. and Green, T. (1985). Species Differences in Response to Trichloroethylene. I. Pharmacokinetics in Rats and Mice. *Toxicol. Appl. Pharmacol.* **79**, 389-400.

Rao, M. and Reddy, J. (1996). Hepatocarcinogenesis of peroxisome proliferators. *Ann. N. Y. Acad. Sci.* **804**, 573-587.

Reddy, J. and Chu, R. (1996). Peroxisome proliferator-induced pleiotropic responses: pursuit of a phenomenon. *Ann. N. Y. Acad. Sci.* **804**.

Rininger, J., Wheelock, G., Ma, X. and Babish, J. (1996). Discordant expression of the cyclin-dependent kinases and cyclins in rat liver following acute administration of the hepatocarcinogen [4-chloro-6-(2,3-xylidino)-2-pyrimidinylthio] acetic acid (WY14,643). *Biochem. Pharmacol.* **52**, 1749-1755.

Roberts, R. (1996). Non-genotoxic hepatocarcinogenesis: Suppression of apoptosis by peroxisome proliferators. *Ann. N. Y. Acad. Sci.* **804**, 588-611.

Roberts, R., James, N., Woodyatt, N., Macdonald, N. and Tugwood, J. (1998). Evidence for the suppression of apoptosis by the peroxisome proliferator activated receptor alpha (PPAR alpha). *Carcinogenesis* **19**, 43-48.

Roberts, R., Nebert, D., Hickman, J., Richburg, J. and Goldsworthy, T. (1997). Perturbation of the mitosis/apoptosis balance: a fundamental mechanism in toxicology. *Fundam. Appl. Toxicol.* **38**, 107-115.

Roberts, R., Soames, A., Gill, J., James, N. and Wheeldon, E. (1995). Non-genotoxic hepatocarcinogens stimulate DNA synthesis and their withdrawal induces apoptosis, but in different hepatocyte populations. *Carcinogenesis* **16**, 1693-1698.

Rokos, C. and Ledwith, B. (1997). Peroxisome proliferators activate extracellular signal-regulated kinases in immortalized mouse liver cells. *J. Biol. Chem.* **272**, 13452-13457.

Rose, M., Germolec, D., Schoonhoven, R. and Thurman, R. (1997). Kupffer cells are causally responsible for the mitogenic effect of peroxisome proliferators. *Carcinogenesis* **18**, 1453-1456.

Rose, M., Rivera, C., Bradford, B., Graves, L., Cattley, R., Schoonhoven, R., Swenberg, J. and Thurman, R. (1999). Kupffer cell oxidant production is central to the mechanism of peroxisome proliferators. *Carcinogenesis* **20**, 27-33.

Rumsby, P., Davies, M., Price, R. and Lake, B. (1994). Effect of some peroxisome proliferators on transforming growth factor-beta 1 gene expression and insulin-like growth factor II/mannose-6-phosphate receptor gene expression in rat liver. *Carcinogenesis* **15**, 419-421.

Sanchez, I. M. and Bull, R. J. (1990). Early Induction of Reparative Hyperplasia in the Liver of B6C3F1 Mice Treated with Dichloroacetate and Trichloroacetate. *Toxicology* **64**, 33-46.

Schoonjans, K., Staels, B. and Auwerx, J. (1996). The peroxisome proliferator activated receptors (PPARS) and their effects on lipid metabolism and adipocyte differentiation. *Biochim. Biophys. Acta* **1302**, 93-109.

Schulte-Hermann, R., Bursch, W., Low-Baselli, A., Wagner, A. and Grasl-Kraupp, B. (1997). Apoptosis in the liver and its role in hepatocarcinogenesis. *Cell Biol. Toxicol.* **13**, 339-348.

Schumann, A. M., Quast, J. F. and Watanabe, P. G. (1980). The Pharmacokinetics and Macromolecular Interactions of Perchloroethylene in Mice and Rats as Related to Oncogenicity. *Toxicol. Appl. Pharmacol.* **55**, 207-219.

Sharma, R., Lake, B. and Gibson, G. (1988). Co-induction of microsomal cytochrome P-452 and the peroxisomal fatty acid beta-oxidation pathway in the rat by clofibrate and di-(2-ethylhexyl)phthalate. Dose-response studies. *Biochem. Pharmacol.* **37**, 1203-1206.

Sher, T., Yi, H., McBride, O. and Gonzalez, F. (1993). cDNA cloning, chromosomal mapping, and functional characterization of the human peroxisome proliferator activated receptor. *Biochemistry* **32**, 5598-5604.

Shiau, A., Barstad, D., Loria, P., Cheng, L., Kushner, P., Agard, D. and Greene, G. (1998). The structural basis of estrogen receptor/coactivator recognition and the antagonism of this interaction by tamoxifen. *Cell* **95**, 927-937.

Shibata, H., Spencer, T. E., Oñate, S. A., Jenster, G., Tsai, S. Y., Tsai, M.-J. and O'Malley, B. W. (1997). Role of Co-activators and Co-repressors in the Mechanism of Steroid/Thyroid Receptor Action. *Recent Prog. Horm. Res.* **52**, 141-165.

Short, R., Robinson, E., Lington, A. and Chin, A. (1987). Metabolic and peroxisome proliferation studies with di(2-ethylhexyl)phthalate in rats and monkeys. *Toxicol. Ind. Health* **3**, 185-195.

Simpson, A. (1997). The cytochrome P450 4 (CYP4) family. *Gen. Pharmacol.* **28**, 351-359.

Soliman, M., Cunningham, M., Morrow, J., Roberts, L., 2nd and Badr, M. (1997). Evidence against peroxisome proliferation-induced hepatic oxidative damage. *Biochem. Pharmacol.* **53**, 1369-1374.

Stauber, A. and Bull, R. (1997). Differences in phenotype and cell replicative behavior of hepatic tumors induced by dichloroacetate (DCA) and trichloroacetate (TCA). *Toxicol. Appl. Pharmacol.* **144**, 235-246.

Stauber, A., Bull, R. and Thrall, B. (1998). Dichloroacetate and trichloroacetate promote clonal expansion of anchorage-independent hepatocytes in vivo and in vitro. *Toxicol. Appl. Pharmacol.* **150**, 287-294.

Sterchele, P., Sun, H., Peterson, R. and Vanden Heuvel, J. (1996). Regulation of peroxisome proliferator-activated receptor-alpha mRNA in rat liver. *Arch. Biochem. Biophys.* **326**, 281-289.

Stott, W., Yano, B., Williams, D., Barnard, S., Hannah, M., Cieszlak, F. and Herman, J. (1995). Species-dependent induction of peroxisome proliferation by haloxyfop, an aryloxyphenoxy herbicide. *Fundam. Appl. Toxicol.* **28**, 71-79.

Stott, W. T., Quast, J. F. and Watanabe, P. G. (1982). The Pharmacokinetics and Macromolecular Interactions of Trichloroethylene in Mice and Rats. *Toxicol. Appl. Pharmacol.* **62**, 137-151.

Styles, J. A., Wyatt, I. and Coutts, C. (1991). Trichloroacetic Acid: Studies on Uptake and Effects on Hepatic DNA and Liver Growth in Mouse. *Carcinogenesis* **12**, 1715-1719.

Templin, M. V., Parker, J. C. and Bull, R. J. (1993). Relative Formation of Dichloroacetate and Trichloroacetate from Trichloroethylene in Male B6C3F1 Mice. *Toxicol. Appl. Pharmacol.* **123**, 1-8.

Tucker, A. N., Sanders, V. M., Barnes, D. W., Bradshaw, T. J., White, K. L., Jr., Sain, L. E., Borzelleca, J. F. and Munson, A. E. (1982). Toxicology of Trichloroethylene in the Mouse. *Toxicol. Appl. Pharmacol.* **62**, 351-357.

Vamvakas, S., Bruning, T., Thomasson, B., Lammert, M., Baumuller, A., Bolt, H., Dekant, W., Birner, G., Henschler, D. and Ulm, K. (1998). Renal cell cancer correlated with occupational exposure to trichloroethene. *J Cancer Res Clin Oncol* **124**, 374-382.

Vamvakas, S., Dekant, W. and Henschler, D. (1993). Nephrocarcinogenicity of Haloalkenes and Alkynes. In *Renal Disposition and Nephrotoxicity of Xenobiotics.*, pp. 323-342, Anders, M. W., Dekant, W., Henschler, D., Oberleithner, H. and Silbernagl, S., Eds., Academic Press, Inc., San Diego, CA.

Van Duuren, B. L., Goldschmidt, B. M., Loewengart, G., Smith, A. C., Melchionne, S., Seildman, I. and Roth, D. (1979). Carcinogenicity of Halogenated Olefinic and Aliphatic Hydrocarbons in Mice. *J. Natl. Cancer Inst.* **63**, 1433-1439.

Wagner, R. L., Apriletti, J. W., McGrath, M. E., West, B. L., Baxter, J. D. and Fletterick, R. J. (1995). A Structural Role for Hormone in the Thyroid Hormone Receptor. *Nature* **378**, 690-697.

Walles, S. A. (1986). Induction of Single-Strand Breaks in DNA of Mice by Trichloroethylene and Tetrachloroethylene. *Toxicol. Lett.* **31**, 31-35.

Wang, T., Cardiff, R., Zukerberg, L., Lees, E., Arnold, A. and Schmidt, E. (1994). Mammary hyperplasia and carcinoma in MMTV-cyclin D1 transgenic mice. *Nature* **369**, 669-671.

Ward, J., Peters, J., Perella, C. and Gonzalez, F. (1998). Receptor and nonreceptor-mediated organ-specific toxicity of di(2-ethylhexyl)phthalate (DEHP) in peroxisome proliferator-activated receptor alpha-null mice. *Toxicol. Pathol.* **26**, 240-246.

Zanelli, U., Puccini, P., Acerbi, D., Ventura, P. and Gervasi, P. (1996). Induction of peroxisomal beta-oxidation and P-450 4A-dependent activities by pivalic and trichloroacetic acid in rat liver and kidney. *Arch. Toxicol.* **70**, 145-149.

Zhou, Y. and Waxman, D. (1998). Activation of peroxisome proliferator-activated receptors by chlorinated hydrocarbons and endogenous steroids. *Environ. Health Perspect.* **106**, 983-988.